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Fifth Interim Report, Colstrip, Montana

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16. ABSTRACT

The EPA has recognized the need for a rational approach to the incorporation of ecological impact information into power facility siting decisions in the northern great plains. Research funded by the Colstrip, Coal-Fired Power Plant project is a first attempt to generate methods to predict the bioenvironmental effects of air pollution before damage is sustained. Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. Since then, key characteristics of the eco-system have been monitored regularly to detect possibe pollution impacts upon plant and animal community structure.

In the summer of 1975, field stressing experiments were begun to provide the data necessary to develop dose-response models for SO_2 stress on a grassland ecosystem. These experiments involve continuous stressing of one acre grassland plots with measured doses of SO_2 during the growing season (usually April through October).

Results of the 1978 field season's investigations are summarized in this publication. The six-year project will terminate in 1980 and a final report will be published after data analyses are complete.

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THE BIOENVIRONMENTAL IMPACT OF A COAL-FIRED POWER PLANT

Fifth Interim Report, Colstrip, Montana
April, 1980

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

The Colstrip, Coal-Fired Power Plant Project is a first attempt to generate methods to predict the bioenvironmental effects of air pollution before damage is sustained. The results will aid planners in assessing the ecological impact of energy conversion activities on grasslands prior to site selection.

Thomas A. Murphy
Director, CERL

PREFACE

The Environmental Protection Agency has recognized the need for a rational approach to the incorporation of ecological impact information into power facility siting and management decisions in the Northern Great Plains. Two capabilities need to be developed. First, in evaluating alternative sites, planners need to be able to predict the kinds and magnitudes of impacts to be expected so that adverse consequences to the environment can be minimized. Secondly, for routine facility management after siting, environmental monitoring methods are needed for detecting incipient ecological damage in time for mitigation efforts to be effective.

Research funded by the Colstrip, Coal-Fired Power Plant Project is a first attempt to generate the methods needed to predict the bioenvironmental effects of SO_2 emissions from coal-fired power plants before damage is sustained. The work can be subdivided into three chronological phases: 1) the identification of information requirements and the expansion of data and information bases to fill these requirements, 2) the integration and synthesis of newly generated data with existing information to define relationships permitting maximum predictive capability, and 3) the provision of information in a format useful to planners and decision makers involved in siting coal-fired power plants.

Research effort for Phase 1 falls roughly into two broad categories: 1) ecological effects monitoring in the vicinity of two 350 megawatt coal-fired power plants at Colstrip, Montana, and 2) field and laboratory process studies designed to elaborate the mechanisms through which coal-fired power plant emissions cause their effects.

Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. This documentation continued until Colstrip generating unit 1 began operation in September, 1975. Since then, key characteristics of the ecosystem have been monitored regularly to detect possible pollution impacts. The current results are in Sections 1-5.

In 1974, a Zonal Air Pollution System (ZAPS) was designed to stress 0.5 hectare areas of native grassland with measured concentrations of SO_2. In the summer of 1975, field stressing experiments were begun to provide the data necessary to develop dose-response models of SO_2 stresses on a grassland ecosystem. A second Zonal Air Pollution System (ZAPS II), was constructed in 1976. Both ZAPS I and ZAPS II were operated during the growing seasons of 1976, 1977, and 1978. The design of these experimental systems has been described in previous interim reports. Their behavior is further described in the present report. Effects on microorganisms, producers and consumers have been monitored throughout the stressing experiments. The results of these experiments to date are summarized in Sections 6 through 22.

The final two sections represent preliminary attempts to utilize information presented in previous sections and previous interim reports to address the project's objectives.

ABSTRACT

The grasslands of the northern plains consist of a complex combination of interacting plant and animal species that capture, store, and utilize energy in a self-sustaining manner. Pollution stress on these rangelands may affect a variety of ecological processes.

The Coal-Fired Power Plant Project at Colstrip is designed to evaluate the environmental impact of air emissions from coal-fired power plants on the Northern Great Plains ecosystems. The project consists of three parts:

(1) A case study of the ecological impact of two 350 megawatt coal-fired generating units at Colstrip, Montana.

(2) A series of field and laboratory process studies designed to elaborate the mechanisms of SO_2 action on grasslands chronically fumigated at low-levels.

(3) Development of a methodology for incorporating ecological effects information into the power plant siting process.

This report summarizes the results of the 1978 field season.

Colstrip Studies

Documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. This continued until Colstrip generating Unit 1 began operation in September 1975. Since then, key characteristics of the ecosystem have been monitored to detect air pollution and its ecological effects.

Ambient air monitoring suggests that background SO_2 levels in the Colstrip, Montana area are approximately 0.1 pphm (2.3 $\mu g/m^3$). In 1978, the average air concentrations, including the power plant contribution, increased to about 0.2 pphm (4.6 $\mu g/m^3$).

The SO_2 exposure at distances beyond five kilometers downwind from the power plant was at least twice that predicted by dispersion models. Also, plume cross sectional measurements at various distances downwind were two or three times wider than predicted by Gaussian diffusion models. Plume trajectories are altered by topography as much as 20° from the direction of prevailing wind.

Both natural aerosols and those generated by the coal-fired power plants at Colstrip, Montana were studied. The man-made aerosols at distances beyond ten kilometers consisted of spherical sulfate-containing particles (0.1-0.4 μm

diameter), natural aerosols of irregularly shaped alumino-silicates (>0.6 μm mean particle diameter), and another man-made aerosol consisting of nitrate-containing droplets (1-2 μm diameter). The nitrate-containing droplets were a significant sink for nitrous oxide formed in the power plant combustion chambers.

Since 1974, plant community monitoring was conducted to collect baseline data at grassland exclosures within 20 kilometers of the coal-fired power plants at Colstrip, Montana. These studies show that grazing deferment effects stabilize gradually over a period of several years. Air pollution effects cannot be adequately assessed until stabilization has occurred. Total canopy coverage is largely determined by graminoid cover which varies substantially among sites. Forb coverage does not vary markedly among sites. Mosses and lichens were uncommon. Shrub cover was determined by pre-treatment distribution. Lichen cover was inversely related to canopy density. Two lichen species, *Usnea hirta* (L.) and *Parmelia chlorochroa*, have shown no adverse effects attributable to Colstrip power plant emissions. Western wheatgrass density varies little within sites but shows significant inter-site variation. Species richness and evenness contribute about equally to species diversity.

Honey bees are excellent scavengers of contaminants in their surroundings. Bees located within 20 kilometers of the power plants accumulated flouride in and on their bodies in 1977 and 1978. Fluoride levels were highest in adult foragers, lower in hive bees, and lowest in the pupa. Arsenic concentrations in or on the bodies of worker honey bees in most cases were less than 0.8 ppm on a dry weight basis for all years (1974-1977). At an apiary 13 kilometers northeast of Colstrip, 1.3 ppm arsenic as observed in 1977. This is twice the mean for 1977 and comparable to levels observed in bees collected near Butte, Montana exposed to arsenic from a copper smelter. Arsenic may concentrate in bees near the smelter to levels 100 times greater than levels seen in bees near Colstrip. These levels appear to cause problems ranging from poor honey production, inability to overwinter, and even death of the bee colony. Other materials such as cadmium also appeared to concentrate in bees.

In summary, the 1978 Colstrip field studies show that the plume from the Colstrip power plants will be dispersed over a larger area with higher downwind SO_2 concentrations than predicted by models. This suggests that a greater than predicted land area may be affected by pollutant deposition and/or attenuation of solar radiation. Pollution effects on the grassland cannot be adequately evaluated until stabilization from grazing deferment occurs. Honey bees accumulate flouride in and on their bodies over time and appear to be valuable bioindicators of plume exposure.

Field and Laboratory Experiments

In 1974, a Zonal Air Pollution System (ZAPS) was designed to stress 0.5 hectare areas of native grassland with measured concentrations of SO_2. Field stressing experiments were initiated during the summer of 1975. A second ZAPS was constructed in 1976. Both ZAPS I and ZAPS II were operated during 1977 and 1978.

Dose characterization on the plots is complex. Seasonal geometric mean SO_2 concentrations were approximately 1, 3, 5, and 9 pphm on the Control, Low, Medium, and High treatment plots, respectively. Daytime SO_2 concentrations are substantially less than nighttime concentrations. Consequently, biologically effective dose rates vary with the diel activity cycle of each species. There is marked vertical stratification of SO_2 in the vegetation canopy at night and slight stratification during the day. Thus, dose rate is also dependent upon the organisms' diel activity patterns in the use of vertical space. Diel SO_2 concentration patterns are similar to those in areas influenced by multiple sources and do not resemble those in the vicinity of the point source at Colstrip.

Western wheatgrass, the dominant grassland species on the ZAPS plots, accumulated sulfur as a linear function of either time or concentration. Young leaves and the youngest portion of the leaves contained less sulfur than their older counterparts regardless of SO_2 treatment. Chlorophyll *a* and *b* concentrations significantly decreased in the absence of visible necrosis. Chlorophyll *a* was more sensitive than chlorophyll *b*. Sensitivity of chlorophylls to sulfur dioxide changed as the growing season progressed indicating cumulative effects and interactions with normal senescence. Western wheatgrass on ZAPS II exhibited significant differences between treatments in the number of roots manifesting necrosis and in their culm dry weight. Low level SO_2 treatment combined with nitrogen fertilization increased herbage yield and altered plant sulfur, nitrogen content, and N:S ratios.

Long term exposure of the grassland has not markedly altered above or belowground biomass dynamics, net primary production, forage quality or species composition. Total living vegetation, total graminoids, western wheatgrass, lichens and perennial species all showed decreasing cover with increasing sulfur dioxide rates. Decomposition rates of western wheatgrass on ZAPS I, High plot decreased by about 10 percent. The decrease was probably due to reduced pH or accumulation of toxic derivatives of sulfur dioxide in the micro-environment of organisms on the decaying leaf surface.

Two lichen species, *Usnea hirta* and *Parmelia chlorochroa*, exhibited reduced photosynthesis rate, disrupted chloroplast structure, and increased algal plasmolysis at the ZAPS sites. Respiration rates and relative chlorophyll extract absorbance were not changed.

Grasshoppers, saprophagous, and necrophagous beetles were reduced in number by increased exposure time and increased SO_2 concentration at the ZAPS sites. A similar decrease was observed in the tardigrade numbers, but the trend was not statistically significant due to the high variability of sample counts. Tardigrades may be sensitive indicators of air pollution effects on soil processes, since they reside near the soil surface where they are more vulnerable to exposure than deeper dwelling soil fauna. Beetles are important processors of carrion and dung.

A laboratory study was conducted to determine the effects of chronic sulfur dioxide exposure on the growth, yield, quality and nitrogen fixation of alfalfa (*Medicago sativa* L.). Sulfur dioxide induced significant reductions in plant growth, yield, and nitrogen fixation in 15 and 20 pphm treatments.

Total sulfur in the forage increased in proportion to the median sulfur dioxide concentrations. Sulfur dioxide caused no significant decreases in forage quality.

Large scale aerial photographs of the ZAPS plots were analyzed visually and densitometrically. A general reduction in density with increased SO_2 exposure was observed in red, green, and blue wave lengths. Photographic density differences were clearly related to SO_2 fumigation. The red density was especially depressed by SO_2 during the active growth season.

In summary, the 1978 ZAPS studies suggest that total living vegetation, total graminoids, western wheatgrass, lichens and perennial species all show decreasing cover with increasing sulfur dioxide exposure rates. SO_2 treatment combined with nitrogen fertilization enhanced herbage yield. Chlorophyll *a* and *b* concentrations in western wheatgrass appear to be sensitive indicators of SO_2 exposure. The decomposition rates of western wheatgrass decreased and grasshopper, beetle, and possibly tardigrade numbers were reduced on various treatment plots. Biomass dynamics of dominant grasses, net primary production, forage quality, and species composition have not been demonstrably altered by SO_2 treatments.

Synthesis

Simulation models of SO_2 deposition and sulfur cycling in a grassland were developed as conceptual tools to organize information. When models were executed simultaneously with others describing primary production, decomposition and abiotic processes, they provided fair representations of the accumulation of sulfur in live and dead leaves and soil. Under simulated exposure to 10 pphm SO_2, many sulfur flows in the model grassland increased causing substantial departure from initial equilibrium.

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SECTION 1

AIR QUALITY MEASUREMENTS IN THE COAL-FIRED POWER PLANT ENVIRONMENT OF COLSTRIP MONTANA

J. D. Ludwick, D. B. Weber, K. B. Olsen, and S. R. Garcia

ABSTRACT

A network of up to six air monitoring and sampling stations were operated (4-23 km) downwind of the 700 MW coal-fired power plants at Colstrip, Montana. Continuous monitoring identified background SO_2 levels in the surrounding air at approximately 0.1 pphm (2.3 $\mu g/m^3$). Average air concentrations measured in 1978 throughout the network increased to about 0.2 pphm (4.6 $\mu g/m^3$) and included the power plant contribution. Meteorological and aerosol correlations with SO_2 concentrations helped to identify and isolate the major source. The high sensitivity for SO_2 measurement provided resolution of all significant power plant plume fumigations at ground level. The magnitude of SO_2 exposures were higher than predicted in this rough terrain environment.

INTRODUCTION

Results are reported from an air sampling and monitoring network operating in the eastern portion of the state of Montana during 1978. This program is an expansion and sophistication of earlier attempts to characterize the air quality downwind of the two coal-fired power plants at Colstrip, Montana (700 MW). A year of continuous operations has yielded data which include significant environmental effects from operation of these power plants 4-23 km downwind. The atmospheric models presently used to estimate pollutant exposures in this rough terrain environment appear to have underestimated the frequency and intensity of power plant emission exposures at the more distant locations.

Long term studies have been reported concerning the downwind ground level concentration of sulfur dioxide in the coal-fired power plant environment (Moore, 1967; Martin and Barber, 1966; Martin and Barber, 1967; Martin and Barber, 1973). In general, these studies have taken place in areas of considerable pollution, where high background levels must be tolerated. Additional problems relating to instrumental sensitivity capabilities, in real time have limited the detection of low level plume fumigation. Lack of instrumental sensitivities may be responsible for high background estimates reported at locations where incoming air appears to have no obvious pollution source (Barnes, 1976). Long distance transport (100 km) of sulfur dioxide from major industrial regions have been reported, however, and the influence of such sources must be considered in some situations (Zeedijk and Velds, 1973). In spite of elevated levels of sulfur dioxide in the background, meteorological correlations have helped identify the larger sources in many instances (Raynor *et al*., 1974, Zanetti *et al*., 1977).

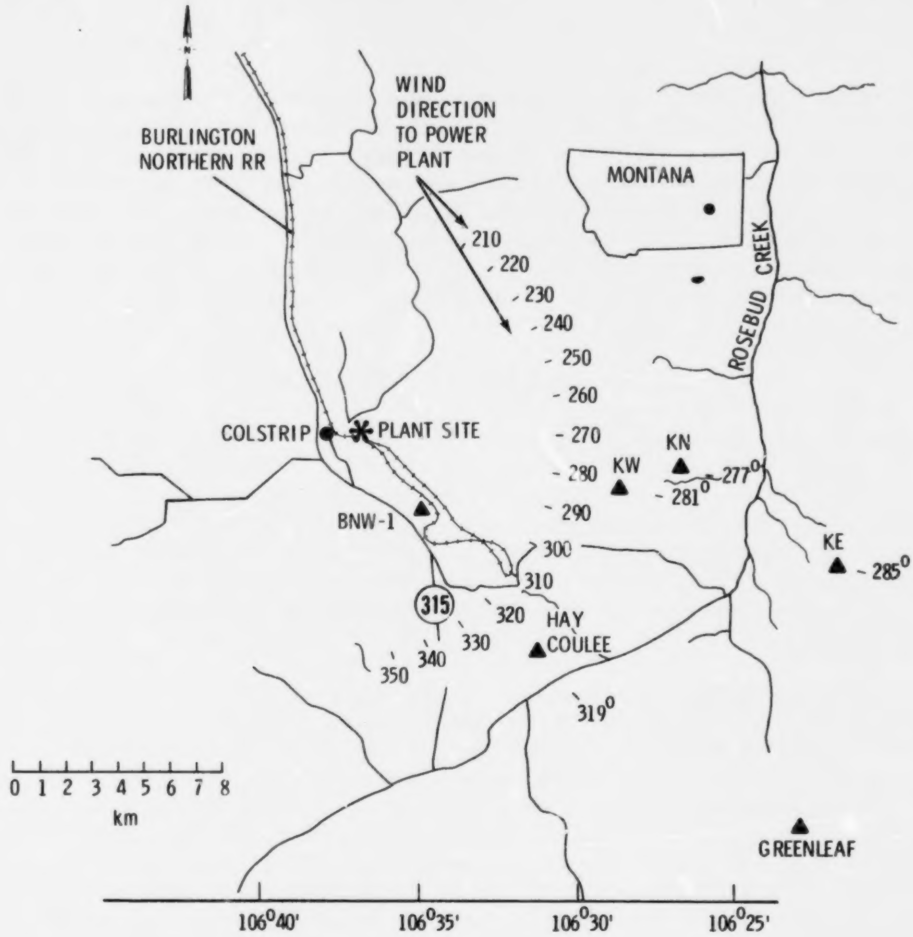
The Great Plains contains a vast reservoir of fossilized energy. The most direct purpose for its use is in supplying the power needs of the region it underlies. It was anticipated that the expanded use of coal for supplying electrical power will present environmental problems which must be addressed. In the Colstrip area a consortium of public utilities have site applications which propose to triple the generating capacity of this area with two additional units. Each of these proposed power plants will have the combined capacity of the two original units. Experimental data such as that reported here, as well as much more, will be necessary to provide accurate siting criteria information on the expected impact of large pollutant emitters. The bulk of the air monitoring and sampling data reported here includes the entire year of 1978.

A review of the collected data illustrates the sensitivity of the air monitoring stations to assess the very low sulfur dioxide air concentrations found in this clean environment. As a result pertinent data are available to estimate the extent of the power plant impact upon the Class 1 region located geographically similar to our more distant monitoring stations.

MATERIALS AND METHODS

The relative positions of the air monitoring stations and the power plant are shown in Figure 1.1. Not more than four sites were operated simultaneously; however, this figure illustrates all locations that were monitored during the course of 1978. The initial site locations selected were based upon a wind-rose analysis of this area. Although all stations were not identical in capability, a typical station description follows.

Air monitoring was conducted on oxides of sulfur utilizing a Meloy Model 285 sulfur analyzer, which was selected for its low noise characteristics. This was particularly significant since the bulk of the ambient data was expected to be below 1.0 pphm (23 $\mu g/m^3$). Ozone levels were measured with a Bendix Model 8002 ambient air analyzer. Particulates were monitored with a Model Rich 100 Environment/One Condensation Nuclei Detector. Ambient air was supplied to the monitoring instrumentation from a common Teflon manifold. High velocity flow was maintained through this manifold. Information from the air monitoring equipment, as well as data from meteorological instrumentation located at the sites, were collected by a microprocessor/datalogger and interfaced to a paper punch tape for storage and computer processing. The meteorological information included windspeed and direction, temperature, humidity and solar radiation. All the equipment was housed in small, highly insulated trailers. Electrical power for most stations was derived from 5.5 kW pro-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106°30'
106°25'

Figure 1.1. Monitoring station location.

pane-fueled generators. The power was divided into two sections: rough power for the heating, air conditioning, and pumps as well as stable power for the instrumentation. An uninterruptible power system supplied highly regulated 115 volt 60 Hz electricity necessary for the stable operation of the air monitoring instrumentation. This equipment also provided power continuity for station operation during periods of short term generator outage. One-thousand gallon storage containers provided a 45-day fuel supply for the generators. This was necessitated by the inaccessibility of some of the stations during the winter months in Montana.

A two-stage cyclone separator was operated in conjunction with the monitoring instrumentation. This provided particulate information which could be correlated with the gas data. The air monitoring data were collected in increments of four minutes and these values were averaged for different pertinent intervals. The data were edited for spurious information and excessive instrumental fluctuations.

A daily quality assurance program for verifying the integrity of the collected air monitoring data was in effect throughout the operation of this program. In addition, Rockwell International, under contract from the Environmental Protection Agency (EPA), performed quarterly audits on a routing basis. The extent of each audit included all operational air monitoring instrumentation and other calibration equipment at the sites visited. A record of the audit reports is available as well as a more complete record of other parameters associated with this report (Ludwick *et al.*, 1979).

RESULTS AND DISCUSSION

The monthly averages of the data from sites that had sufficient on-line time are shown in Tables 1.1, 1.2, 1.3, and 1.4. The concentrations of sulfur dioxide are quite low, with respect to the urban environment. Except for individual short-term events discussed later, the impact of oxides on nitrogen from the power plant upon the ozone averages would be masked by the ubiquitous ambient levels of this species. In general, the levels of ozone are consistent with those observed in other relatively clean environments (Ludwick *et al.*, 1976).

The concentration fluctuations of gaseous sulfur at the stations is sufficient in some instances to be of interest. For example, in March the averaged sulfur dioxide concentrations at Hay Coulee, 12 km from the power plant was 0.56 pphm. This represents a contribution of almost 0.05 pphm on an annually averaged basis from this single month. The Northern Cheyenne Indian Reservation extends to within 22 km of the power plant, in the same general direction as Hay Coulee. This land bears a Class 1 air quality designation. With reference to Table 1.5, the Air Quality Standards, we see that an elevation of no more than 0.07 pphm is acceptable before significant air quality deterioration is suggested. Since values of this magnitude were not expected from EPA models utilized in this area, the question of the sulfur dioxide source and downwind dilution between 12 and 22 km becomes rather important.

No reliable ambient sulfur dioxide background measurements are available prior to power plant operation. These data suggest that averaged present day background levels decline to about 0.07 pphm and are typically about 0.1 pphm (2.31 $\mu g/m^3$). Values of this magnitude correspond to those observed in the cleanest areas of industrialized nations (Junge, 1963). Assuming this to be a reasonable conservative minimum, the magnitude of the effect from a pollution source can then be considered. Lower background estimates would, of course, infer more potent anthropogenic sulfur dioxide sources. A summation and averaging of this data on an annual basis indicate that both KE and KW stations observed an increase of 0.08 pphm (1.9 $\mu g/m^3$) even though KE is several kilometers further downwind of the Colstrip area. A similar increase was observed at BNW-1, very close to Colstrip. A slightly larger increase of 0.12 pphm (2.66 $\mu g/m^3$) was observed at Hay Coulee, downwind of the BNW-1 site. Although variations in operational periods exist, it appears that an overall elevation of 0.1 pphm of sulfur dioxide throughout the area were measured during 1978.

TABLE 1.1. HAY COULEE MONTHLY AVERAGES

Date	Wind Speed kmph	Relative Humidity %	Temperature °C	Ozone pphm	SO_2 pphm
2/78	7.0	70.9	-11.2	4.3	0.37
3/78	7.0	61.3	-2.4	4.2	0.36
4/78	12.7	54.6	8.2	3.9	0.18
5/78	10.8	68.2	12.6	4.1	0.13
6/78	9.5	56.6	19.4	4.0	0.23
7/78	9.0	52.4	24.1	4.3	0.16
8/78	9.2	43.0	22.3	3.3	0.10
9/78	9.3	56.0	15.8	2.8	0.15
10/78	7.7	50.0	7.3	2.7	0.17
11/78	5.7	61.5	-8.7	3.3	0.15
12/78	9.5	55.4	-13.0	3.4	0.17

TABLE 1.2. KLUVER EAST MONTHLY AVERAGES

Date	Temperature °C	Ozone pphm	SO_2 pphm
3/78	8.9	4.0	0.18
4/78	9.1	3.9	0.18
5/78	12.3	4.5	0.22
6/78	16.5	3.9	0.34
7/78	27.4	4.6	---
8/78	22.3	3.6	0.14
9/78	18.9	3.0	0.09
10/78	12.3	3.2	0.07

Evidence as to the source of the pollution comes from several areas throughout this report. Figure 1.2 illustrates the generally correlating character of the monthly sulfur dioxide fluctuations of the individual stations. This is indicative of a common pollution source influencing the area.

TABLE 1.3. KLUVER WEST MONTHLY AVERAGES

Date	Temperature °C	Ozone pphm	SO_2 pphm
3/78	7.0	4.0	0.19
4/78	8.5	3.9	0.16
5/78	12.8	4.4	0.15
6/78	18.8	4.1	0.26
7/78	21.3	4.3	0.14

TABLE 1.4. BNW-1 MONTHLY AVERAGES

Date	Wind (Degrees)	Temperature °C	Ozone pphm	SO_2 pphm
7/78	--	24.3	4.5	0.20
8/78	--	24.0	4.1	0.14
9/78	229	17.2	3.4	0.14
10/78	250	10.8	3.4	0.13
11/78	231	-3.5	3.6	0.20
12/78	257	-5.4	3.6	0.26

In addition to the longer, time averaged values mentioned, many interesting features of the monitoring information are apparent in the real time data. Due to the remote rural location of the stations, most of the air being monitored contains little more than hemispheric concentrations of gaseous and particulate materials. When meteorological conditions prevail that are conducive to downward transport of the power plant plume to the stations, plume strikes are unmistakably prominent features upon this data base. Figure 1.3 is a classical plume strike situation in which elevated levels of sulfur dioxide are correlated with particulates from the power plant stack when the wind vector orients from the plant direction. Excesses of nitric oxide in the plume from high temperature coal burning processes are often apparent in the data as a result of their interaction with atmospheric ozone, as follows:

$$O_3 + NO \rightarrow NO_2 + O_2 \quad (3)$$

TABLE 1.5. SULFUR DIOXIDE AIR QUALITY STANDARDS

		Averages		
		Annual	24 Hour	3 Hour
Federal*				50.0 pphm (1300 μg/m^3)
EPA (Classified Areas) Prevention of Significant Deterioration				
Class 3	Industrial	3.0 pphm	14.0 pphm	50.0 pphm
Class 2	Most of USA	5.7 μg/m^3	38 μg/m^3	267 μg/m^3
Class 1	Pristine (Natl. Parks, Wilderness Indian Lands)	0.7 pphm (2 μg/m^3)	1.9 pphm (5 μg/m^3)	9.5 pphm (25 μg/m^3)
State of Montana		20 μg/m^3	100† μg/m^3	250†† μg/m

* Not to be exceeded (NTBEX) more than once per year.
† NTBEX - 1 day/3 month period.
†† 1 hr average NTBEX - 1 hr/4 consecutive months

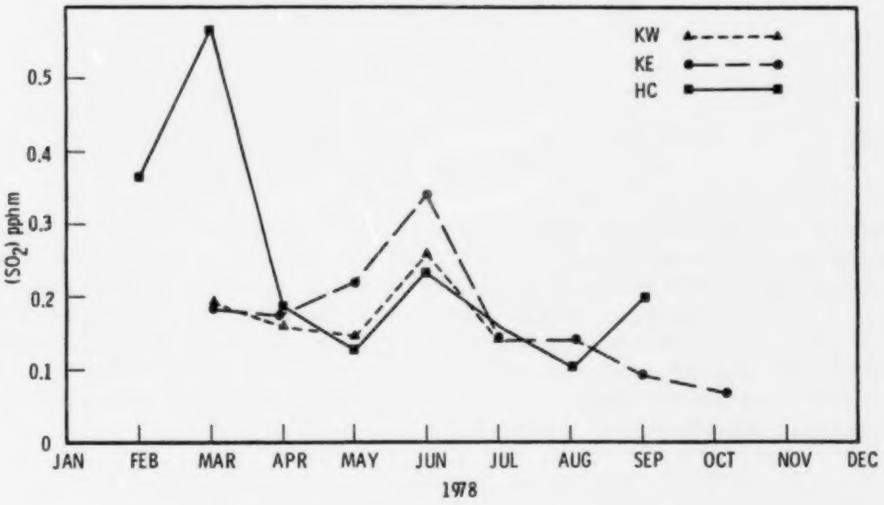

Figure 1.2. Monthly SO_2 correlations between air monitoring stations.

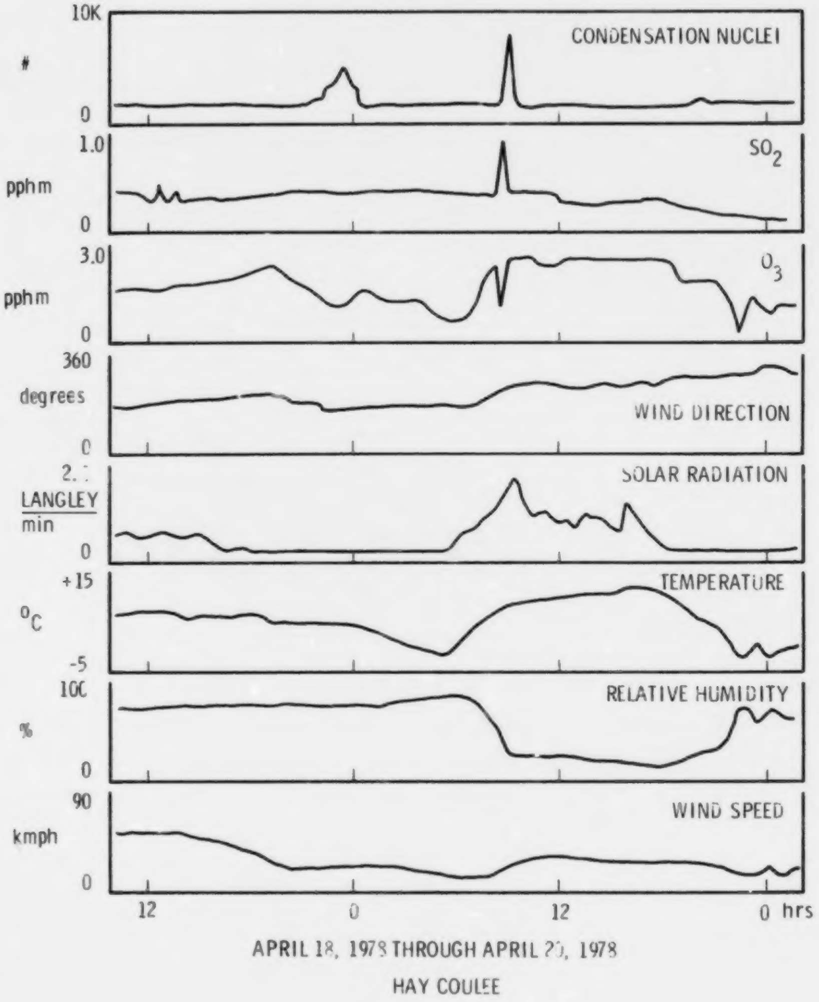

Figure 1.3. Air monitoring information: typical plume strike.

The anticorrelations between sulfur dioxide and ozone are further evidence of the presence of the power plant plume. Not all sulfur plume strikes bear such evidence, however. Sulfur dioxide excursions are occasionally accompanied by unchanged or increasing ozone concentrations. Masking of the ozone anticorrelation can take place in several ways. At first, while consuming ozone, NO is converted to NO_2 and adds to that already emitted. A second process, creating ozone, is known to become important during daytime hours as the concentration of NO_2 increases (Davis *et al.*, 1974):

$$NO_2 \xrightarrow{h\nu} NO + O^{\cdot} \qquad (2)$$

$$O^{\cdot} + O_2 \longrightarrow O_3 \qquad (3)$$

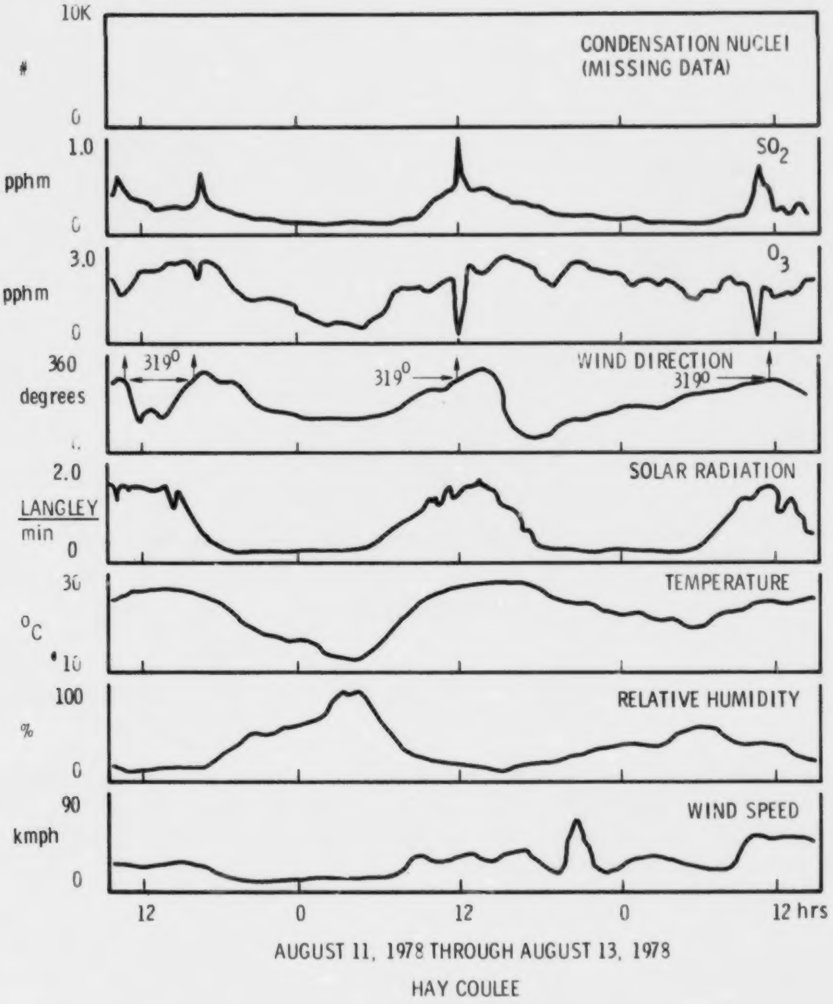

Figure 1.4 Air monitoring information: multiple plume strikes.

Thus, the age of the pollutant plume may influence the net effect of NO and NO_2 on the correlating ozone concentrations.

Elevated ozone concentrations have also been observed above stable underlying air masses (Miller, 1974; Ludwick *et al.*, 1976). Convective turbulence, which appears important in transferring the pollutant plume to the monitoring station may also, on occasion, be responsible for elevated ozone levels and interference with anticorrelations that were initially created by the pollution source.

A 2-day period is illustrated in Figure 1.4 in which three plume strikes were evident each time the wind cycled through the plant direction. It is apparent from the frequency of appearance of these conditions that plume strikes

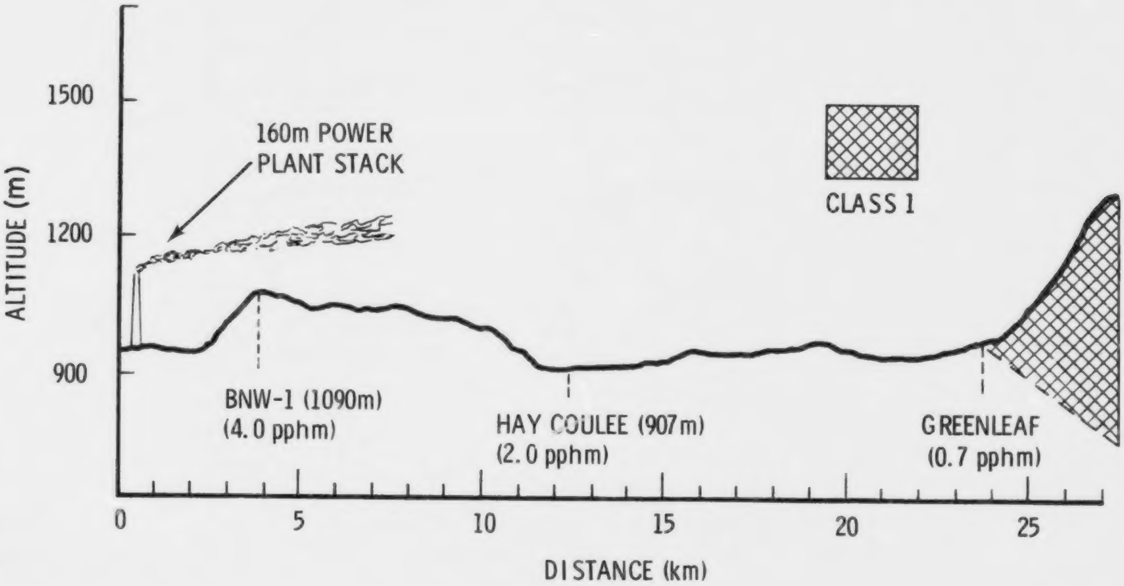

Figure 1.5. Southeast terrain cross section from the Colstrip power plants.

are rather common (376 individual plume strikes were observed throughout the monitoring network in 1978, Ludwick *et al.*, 1979). Daytime convective turbulence as suggested by fluctuations in the solar data, appears often to mix the plume downward to the stations in spite of the elevated plume injection height and interfering terrain.

As field experience was gained concerning the probable location of plume intercepts, emphases were placed on relocating the monitoring stations for maximum effectiveness. The results of such reorientation are shown in Figure 1.5. Here, three co-linearly oriented stations (see Figure 1.1) exhibited the effects from the same power plant plume. It is also a representation of the topographical features southeastward from the Colstrip, Montana area. The low peaks to the east are slightly south of the exact station orientation. Although Class 1 land falls within the Greenleaf site distance, it does not approach this site in its actual geographic fluctuations. The maximum concentrations observed at each site are listed. This was one of two similar triple strike situations that took place in early November 1978. At that time, approximately 5,000 pphm (1150 mg/m^3) of sulfur dioxide were measured in the output from each of two power plant stacks. Thus, the concentration dilution from the first sample location 3 km downwind was approximately 2.5 x 10^4.

Previous estimates of the frequency and intensity of plume fumigations at the more distant monitoring stations were negligible. This was not found to be the case. These estimates were derived from diffusion models which attempted modification of existing flat surface models to the mountainous terrain environment. A brief attempt was made, under the auspices of this program, to compare downwind exposure predictions from the diffusion models that are in present usage, with those actually observed at the sites. Experience with the EPA, PTMTP atmospheric model used for comparison purposes during high sulfur dioxide exposures in February and March indicated a considerable underestimation of the observed values. Use was made, therefore, of a computer modeling program at the University of Montana relied upon for downwind plume estimates (Williams and Tourangeau, unpublished work) in this type terrain. Atmospheric stability data was incorporated into this model derived from tower instrumentation located beside the power plant stack. Comparisons between actual and predicted concentrations of sulfur dioxide at the sites in Figure 1.5 are shown on Table 1.6. The model predictions were based upon maximum exposures from full power operation of the coal-fired plants. This was not the situation at this time; however, even these overestimates fall short of reality at the greater distances. Also evident in this case history is the variation of exposure with distance. Although the peak concentrations observed at the sites varied by a factor of 5, the integrated exposures did not change significantly with downwind travel. This produced unexpectedly high exposures at longer distances. Meteorological situations that promote such occurrences are evidently more frequent than expected.

TABLE 1.6. DIFFUSION MODEL PREDICTIONS vs. MEASURED HOURLY AVERAGED (SO_2)

	Time	BNW-1	HC	Greenleaf
Model†	9 - 1000	0 pphm	0 pphm	0 pphm
	10 - 1100	0.11	0.02	0.27
	11 - 1200	0.01	0	0.01
	12 - 1300	2.2	1.0	0.05
Measured*	9 - 1000	0.25	0.35	0.25
	10 - 1100	0.50	0.50	0.30
	11 - 1200	0.30	0.60	0.45
	12 - 1300	0.30	0.45	0.50

† See (Williams and Tourangeau, unpublished work).
* All values / 0.05 pphm.

The 1978 data set was interrogated with respect to 3-hr and calendar day sulfur dioxide exposures. These limits were used to allow comparisons with the shorter term National Air Quality Standards (Table 1.5). No excesses were observed for the Class 2 category on which the sites were located. The total number of estimated excesses with respect to the nearby Class 1 land are shown in Table 1.7 along with the other pertinent data. A specific compilation by date, time and exposure is available for all the monitoring sites (Ludwick *et al.*, 1979). A significant number of these days are illustrated in the monitoring information. Because of the close proximity of the sites to the Class 1 area, their importance is enhanced.

In order to identify the location of probable sources of sulfur dioxide to each major monitoring station, the direction of incoming sulfur dioxide was correlated with its concentration isopleth. The entire year of data was

TABLE 1.7. FREQUENCY OCCURRENCE OF ELEVATED SO_2 EXPOSURES DURING 1978

Site	Distance From Source (km)	3 hr AVE $\geq$ 10 pphm	24 hr AVE $\geq$ 2 pphm	Observation Period (mo)
BNW-1	4.1	0.9	7.2	5.5
Kluver West	11.2	0.5	5.4	4.5
Hay Coulee	11.6	1.8	16.1	10.8
Kluver North	14.1	0	1.9	1.8
Kluver East	18.2	0	4.7	6.3
Class 1 Land	22.2	----	1.2	12*
Greenleaf	22.5	0	0.1	1.0

* Estimated number of excesses of air quality standards as interpolated from graphic analysis of sulfur concentration vs. distance from the power plant.

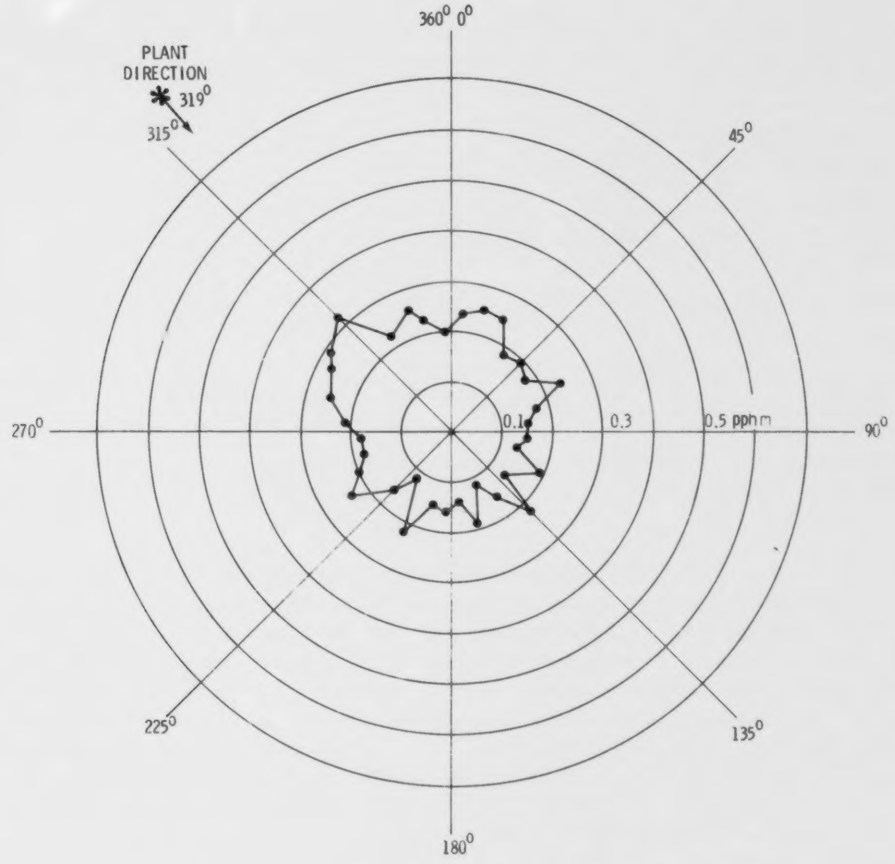

Figure 1.6. Time averaged SO_2 concentration isopleth for 10° increments around Hay Coulee for 1978.

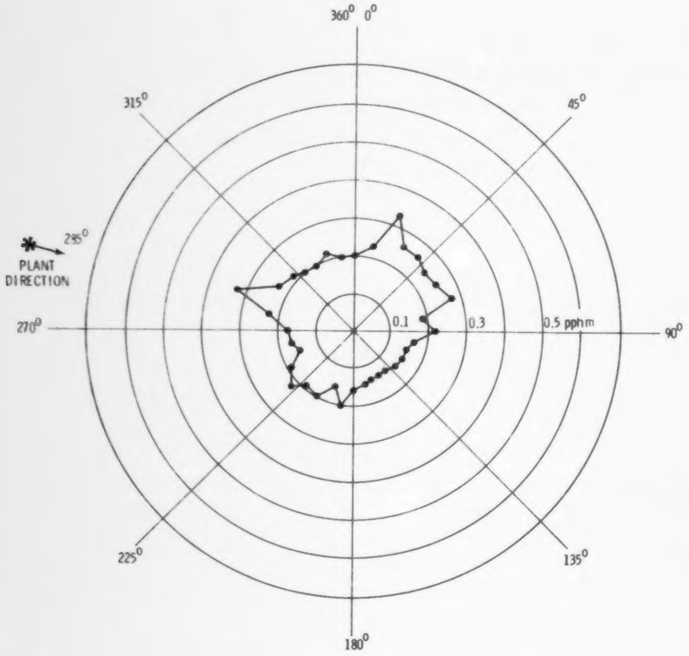

Figure 1.7. Time averaged SO_2 concentration isopleth for 10° increments around KE for 1978.

Figure 1.8. Time averaged SO_2 concentration isopleth for 10° increments around KW for 1978.

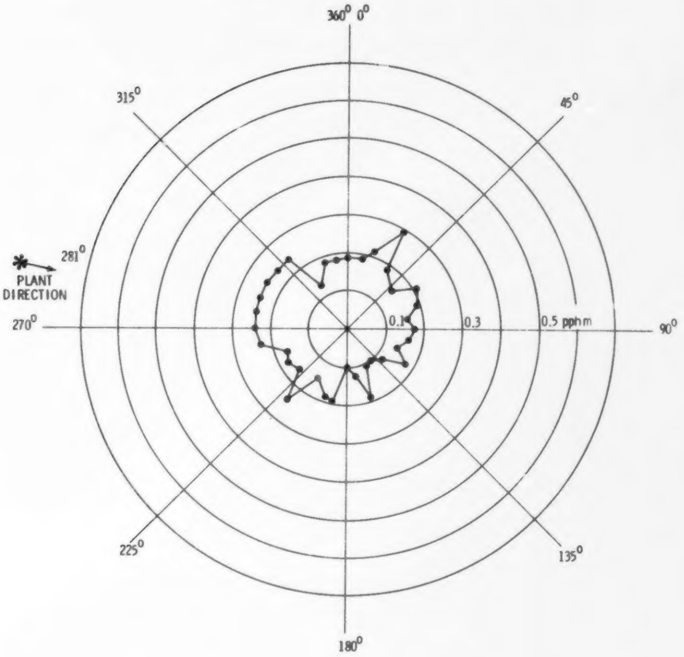

TABLE 1.8. FREQUENCY DISTRIBUTION OF SO_2 EXPOSURES IN THE AIR MONITORING NETWORK

Hourly Averaged SO_2 (pphm)	Number of Times Air Concentration Exceeded or Equaled					
	Hay Coulee	KE	KW	KN	BNW-1	Greenleaf
*0	4638	1987	1926	821	3041	334
0.1	3035	1319	1339	386	1732	80
0.2	2408	1006	1040	297	1291	46
0.3	1625	660	676	196	846	22
0.4	944	368	274	123	537	8
0.5	577	180	103	59	297	1
0.6	320	57	39	21	168	0
0.7	194	23	22	7	94	0
0.8	125	11	15	1	66	0
0.9	90	4	11	0	49	0
1.0	43	3	5	0	37	0
1.1	28	1	5	0	27	0
1.2	19	1	4	0	23	0
1.3	15	1	2	0	15	0
1.4	12	1	1	0	11	0
1.5	9	1	1	0	8	0
1.6	7	1	1	0	8	0
1.7	6	1	1	0	6	0
1.8	1	1	1	0	6	0
1.9	0	1	0	0	6	0
2.0	0	1	0	0	5	0
2.1	0	1	0	0	4	0
2.2	0	1	0	0	3	0
2.3	0	1	0	0	3	0
2.4	0	1	0	0	3	0
2.5	0	0	0	0	3	0
2.6	0	0	0	0	3	0
2.7	0	0	0	0	2	0
2.8	0	0	0	0	1	0
.	.	.	.	.	.	.
.	.	.	.	.	.	.
5.0	0	0	0	0	1	0

* Valid data collection times.

utilized for this purpose in order to reflect all possible sources of sulfur dioxide. Figures 1.6, 1.7, and 1.8 illustrate the results of these comparisons. Each display is a sulfur dioxide concentration isopleth on an annual basis for 10° directional increments around the particular stations shown. Several features are common to these illustrations. Each has a major concentration lobe in the direction of the coal fired power plants. All illustrate a rather low sulfur dioxide input from southeasterly winds opposed to the power plant's direction. A lesser source term seems apparent to the northeast between 15° and 30° true north with respect to each station. A comparison of the topographical features of the area with this secondary source was helpful. It appears that the apparent direction of this secondary source correlates with the contours of Rosebud Creek. Evidently the common westerly winds fumigate the area to the north of our sites. Then sulfur dioxide becomes trapped in the lower terrain and northerly winds create flow through the valley bringing elevated concentrations of sulfur dioxide to the stations.

As the plant plume moves eastward over the station network, it will from time to time, as shown earlier, mix downward to ground level. The frequency of this occurrence as well as the magnitude of such events relate to the instantaneous and integrated exposures observed from the emitted pollutants. Table 1.8 illustrates the integrated hourly sulfur dioxide exposure frequency actually observed at the sites. This data is presented in a manner comparable with other field studies (Drufuca and Guigliano, 1977; Zimmer and Jutze, 1964). These studies suggest that the product of the sulfur dioxide concentration and exposure time should be correlated with biological damage that might occur from such fumigations. The period of operation varied considerably from site to site as instrumentation was utilized in what appeared to be the most expeditious manner at the time. Data omission also occurred because of power failures, instrument breakdowns and data editing.

Aerosol Sampling Data

An aerosol sampling program was conducted at three of the sites in conjunction with the air monitoring. Samples were collected at intervals of a day or longer depending upon weather and personnel availability. The sampling system consisted of a cyclone separator with special orifice and pump. The systems were matched to provide an aerosol separation at slightly less than one micron. Calibration was accomplished using standardized aerosol dispersions. Two fractions were collected from each sample with the fine portion accumulating on Whatman 41 filter paper. The larger fraction was centrifugally maintained in a stainless cup of the separator. A procedure was developed for slurrying this fraction and transferring it onto a polypropylene film. Then both fractions underwent x-ray fluorescence analysis for a series of trace elements. Thin film standards were routinely utilized in each batch analyzed by this technique.

The entire 1978 data set was scanned for level sulfur dioxide concentrations. These are called plume "strike days" and are defined as those in which the hourly averaged sulfur dioxide concentrations exceeded 1.0 pphm (23 $\mu g/m^3$). It was also noted that this situation occurred only when the wind direction was from the power plants. A second set of background days of "non-strike days" were also derived from the data. These were days in which the winds blew from the power plant quadrant but the sulfur dioxide levels were less than 0.2 pphm (4.6 $\mu g/m^3$). This method provided a means of comparing the influence of the plume upon the aerosol.

By comparing the elemental distributions of samples collected during these two periods, one can look for changes in the Colstrip natural aerosol levels with the addition of the power plant plume. Table 1.9 illustrates results of this comparison and includes background and source term information taken from earlier measurements in the Colstrip area (Crecelius *et al.*, 1976).

TABLE 1.9. ELEMENTAL DISTRIBUTIONS IN COLSTRIP AREA AEROSOLS

	Ratio in Sample/Ratio in Crustal*			
Element	Non Strike Days	Stack	Strike Days	Colstrip Soil
Ca	1.3	26	1.8 ± 6%	1.4
Ti	1.0	8	1.3 ± 17%	1.4
Mn	0.80	4.8	1.1 ± 32%	1.0
Fe	1	1	1 ± 2%	1
Zn	19	7	97 ± 11%	3.4
Se	1000	26,000	9000 ± 39%	600
Pb	68	68	450 ± 11%	-
As	90	350	825 ± 31%	9.3
K	1.1	0.1	1.6 ± 10%	1.7
Br	128	244	990 ± 8%	3

* All values were normalized to Fe.

The ratios on the table were derived by first normalizing each element to the amount of iron found in the sample in question and then dividing that number by the elemental ratio observed in normal crustal material. The stack column illustrates the relative potency of the power plant emissions to change the natural aerosol distribution. The Colstrip aerosol column illustrates the aerosol differences that already exist between eastern Montana and average crustal distributions of elements. It appears that the selenium ratios had been modified in the aerosol by the stack; however, definitive explanations of other elemental changes are not as evident. The elevated lead and bromine values reflect the influence of Colstrip traffic upon the aerosols since the stack is incapable of modifying the ratios to the extent shown. The effects of mining operations in the plant direction versus those in other directions would also leave their imprint upon the particulate data although no such directional correlation was observed. In most situations, contributions to the aerosol come from several sources including the natural crustal materials and exact definition is complex. Significant elemental enrichment of the aerosol versus local soil were found for arsenic (26x) and Zn (3.5x) in background measurements of the Colstrip area prior to power plant startup (Crecelius *et al.*, 1976).

Utilizing the definition of strike and non-strike days given previously, Table 1.10 illustrates the ratios found between the fine and coarse particulate for each element in the aerosols. Those elements usually associated with crustal materials appear equally distributed between fractions. Elements whose volatility or ability to fractionate to smaller particulate are found on the smaller fraction.

TABLE 1.10. AEROSOL SIZE DISTRIBUTIONS IN SO_2 POLLUTED AIR ON STRIKE DAYS

Ratio of Fine/Coarse (<1μ/>1μ)			
Ni	8 ± 6	Tl	0.13 ± 0.10
Cr	9 ± 5	Ca	0.86 ± 0.66
As	17 ± 6	Mn	0.69 ± 0.31
Zn	20 ± 4	K	0.69 ± 0.21
Pb	23 ± 14	Fe	1.3 ± 1.1
Se	28 ± 31		
Br	136 ± 81		

CONCLUSION

The pristine environment of Montana State lent itself to identifying, in part, the downwind impact from operation of the first major coal-fired power plants in that region. The compexity of contributing sources in the industrialized areas make specific plant impact relationships difficult to assess. For example, results from a monitoring program of similar orientation around two much larger plants (3000 MW) indicate that their combined effect represents only a 10% sulfur dioxide increase in the seasonal background levels (Martin, 1973), whereas, our study showed that winds from the power plant bring greater than twice the sulfur dioxide concentration to the monitoring network as from other directions. Although the Colstrip power plant plumes have been clearly identified, the quantitative levels of sulfur dioxide are very low. With respect to both exposures and maximum observable concentrations, we certainly are dealing in the parts per billion range.

Prior estimates of the extent of downwind sulfur dioxide fumigation were negligible with respect to the data presented here. In all specific cases studied beyond five kilometers, the actual exposures were at least twice that predicted by field models in use, and generally more. Monthly averaged data as well as individual case situations indicate that although peak exposures may change dramatically with distance, the integrated exposures do not. The atmospheric models do not suggest this and, therefore, diverge from the measured values with distance. The high monthly values measured 12 km from the power plant at Hay Coulee may well represent approximate exposures further downwind. These data strongly suggest longer-range monitoring information is necessary to improve power plant siting criteria.

The biological impact of the power plants has been under study for some time. Results from such work are reported on a continuing basis from the Corvallis, EPA (Preston and Gullett,1979). Three of the monitoring stations were co-located within rangeland plots that were set aside specifically for assessing biological changes in the power plant environment. Subtle effects on the biota, with time, have been noted in the experimental data prior to and during our monitoring program. These may now be compared with our data to determine if correlations with long-term low-level chronic sulfur dioxide exposures exist.

REFERENCES

Barnes, R. A. 1976. Long-Term Mean Concentrations of Atmospheric Smoke and Sulfur Dioxide in Country Areas of England and Wales. Atmospheric Environment, 10:619-631.

Crecelius, E. A., J. C. Laul, L. A. Rancitelli, and R. L. McKeever. 1976. Baseline Air Quality Data from Colstrip, Montana. Pacific Northwest Laboratory Annual Report for 1975 to the USERDA Division of Biomedical and Environmental Research, Part 3, Atmospheric Sciences, BNWL-2000 PT3 Battelle-Northwest, Richland, WA. pp. 25-26.

Davis, D. D., G. Smith, and G. Klauber. 1974. Trace Gas Analysis of Power Plant Plume via Aircraft Measurements: O_3, NO_x, and SO_2 Chemistry. Science, 186:733-736.

Drufuca, G., and M. Guigliano. 1977. The Duration of High SO_2 Concentrations in an Urban Atmosphere. Atmospheric Environment, 11:729-735.

Junge, C. E. 1963. Air Chemistry and Radioactivity. Academic Press, New York.

Ludwick, J. D., T. D. Fox, and L. L. Wendell. 1976. Ozone and Radionuclide Correlations in Air of Marine Trajectory at Quillayute, Washington. J. Air Pollut. Control Assoc., 26:565-569.

Ludwick, J. D., D. B. Weber, K. B. Olsen and S. R. Garcia. 1979. Air Quality Measurements in the Coal-Fired Power Plant Environment of Colstrip, Montana. Pacific Northwest Laboratory, PNL 2964, Battelle-Northwest, Richland, WA.

Martin, A. and F. R. Barber. 1966. Investigation of Sulfur Dioxide Around Modern Power Stations. J. Inst. Fuel, 39:294-307.

Martin, A. and F. R. Barber. 1967. Sulfur Dioxide Cocentrations Measured at Various Distances from a Modern Power Station. Atmospheric Environment, 1:655-677.

Martin, A. and F. R. Barber. 1973. Further Measurements Around Modern Power Stations—I-III. Atmospheric Environment, 7:17-37.

Miller, A. 1974. Rural and Urban Ozone Relationships. J. Air Pollut. Control Assoc., 24:819-821.

Moore, D. J. 1967. SO_2 Concentrations Measurements Near Tilbury Power Station. Atmospheric Environment, 1:389-410.

Preston, E. M. and T. L. Gullett. 1979. The Bioenvironmental Impact of a Coal-Fired Power Plant. Fourth Interim Report, Colstrip, Montana, EPA-600/3-79-944, U.S. Environmental Protection Agency, Corvallis, Oregon.

Raynor, G. S., M. E. Smith, and I. A. Singer. 1974. Meteorological Effects of Sulfur Dioxide Concentrations on Suburban Long Island, New York. Atmospheric Environment, 8:1305-1320.

Williams, M. D. and P. C. Tourangeau. Unpublished Work.

Zanetti, P., P. Melli, and E. Runca. 1977. Meteorological Factors Affecting SO_2 Pollution Levels in Venice. Atmospheric Environment, 11:605-616.

Zeedijk, H., and C. A. Velds. 1973. The Transport of Sulfur Dioxide Over a Long Distance. Atmospheric Environment, 7:849-862.

Zimmer, C. E. and G. A. Jutze. 1964. An Evaluation of Continuous Air Quality Data. J. Air Pollut. Control Assoc., 14:262-266.

SECTION 2

PLUME AND AEROSOL PROPERTIES NEAR COLSTRIP

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ABSTRACT

Analysis of plume aerosol samples with the transmission electron microscope has shown that the small-size fraction (~0.2 μm diameter) is dominated by sulfate particles. The samples included a few nitrate-containing particles of diameter 0.4 to 1.0 μm, but most of the nitrate-containing particles were found in the diameter range of 1-2 μm.

Measurement of the plume cross-sectional dimensions with the research aircraft has shown that the smoke plume is often wider than predicted by Gaussian diffusion models. The vertical extent as measured was less than the predicted value, however. Tracking of the plume with the aircraft to distances up to 50 km downwind has shown that the plume may be diverted as much as 20° from the prevailing wind direction by terrain features.

INTRODUCTION

In May 1975 the Atmospheric Chemistry Program Area (ACPA), Atmospheric Physics and Chemistry Laboratory, with the Atmospheric Spectroscopy Program Area (ASPA), Wave Propagation Laboratory, began a research program at the Hay Coulee site, 12 km southeast of Colstrip, Montana. The work was designed to characterize the aerosol content of the Colstrip power plant plume and its environs. The ACPA and ASPA mobile laboratories were operated at the Hay Coulee site during May-June and August-September of 1975, 1976 and 1977; the ASPA laboratory was moved to the Battelle No. 1 site for the last week of the August-September 1977 period to take advantage of the line-of-sight view of the plume at the point of exit from the power plant smoke stack. The information from these field studies has been reported (Abshire *et al.*, 1978; Van Valin *et al.*, 1979).

When its mobile laboratory was in use at Hay Coulee, ACPA also operated its research aircraft in the vicinity of Colstrip, beginning in August 1975. There were additional aircraft field trips in February 1977, June 1978, and February and March 1979. Information from the aircraft operation during 1975 was reported in the Third Interim Report (Abshire *et al.*, 1978); a partial report of the 1976 and 1977 projects was included in the Fourth Interim Report (Van Valin *et al.*, 1979). This report includes some unevaluated aspects of the 1976 and 1977 projects and some information from the 1978 and 1979 projects, e.g., plume trajectory and diffusion, fine particle formation, gas reactions, and secondary aerosol characteristics.

From 1975, the Colstrip research project was carried out concurrently with other research projects related to energy production at the Four Corners power plant near Farmington, New Mexico, at the Kennecott Copper Company near Salt Lake City, Utah, in the vicinity of the petroleum-refining complex in the south Los Angeles basin, and recently, in the vicinity of the Homer City, Pennsylvania, power plant. The findings from the study of the plume of the Four Corners and Colstrip power plant plumes, particularly in rate and type of secondary particle formation, in spite of considerable differences in plant capacity, emissions control methods, siting, and climate.

The Four Corners power plant production capacity is about 3 times that of the Colstrip plant. Four Corners uses five generating units; emissions from the two largest units, 800 MW each, are controlled by electrostatic precipitators, and from the other three units by lime scrubbers. Research findings from the Four Corners plant follow: (1) only 2 percent (by mass) of the particulate emission was water soluble and contained the elements S, Na and Ca, whereas the 98 percent that was insoluble contained the elements Si, Al, and Fe (Parungo *et al.*, 1978a); (2) sulfur is preferentially concentrated at the surface of 40 percent to 60 percent (by number) of the fly ash particles (Parungo *et al.*, 1978a); (3) the sulfur-containing layer on fly ash consists of ammonium sulfate or bisulfate, and the thickness of the layer is inversely related to the diameter of the fly ash particle (Mamane and Pueschel, 1979a); (4) the number of secondary particles formed by a gas-to-particle conversion process is far greater than the number of primary (fly ash) particles (Pueschel and Van Valin, 1978), and (5) Mamane and Pueschel (1979a) concluded that long-range transport of both sulfur dioxide and sulfates occurs downwind from the power plant.

DISCUSSION AND RESULTS

Aerosol Composition

Nuclepore filter samples were used for the determination of ice nucleus concentration and for elemental composition determination, by the scanning electron microscope with energy dispersive X-ray analysis (SEM-EDX). The histograms in figures 2.1a-2.1e, show elemental occurrence, shape, and mineral type of samples collected during August-September 1977. Figures 2.2a-2.2c represent samples collected during February and March 1979; they were analyzed for 22 elements. Figures 2.2b and 2.2c include relative signal strength, indicating elemental abundance as well as distribution. Thus, in Figure 2.2b, where 47 percent of the emitting particles contain Si, the X-ray emissions due to Si account for 34.5 percent of all X-ray emissions; 71.5 percent of emitting particles contain Ca, but Ca emissions account for only 14.7 percent of total emissions; and 9.4 percent of emitting particles contain Fe, but Fe

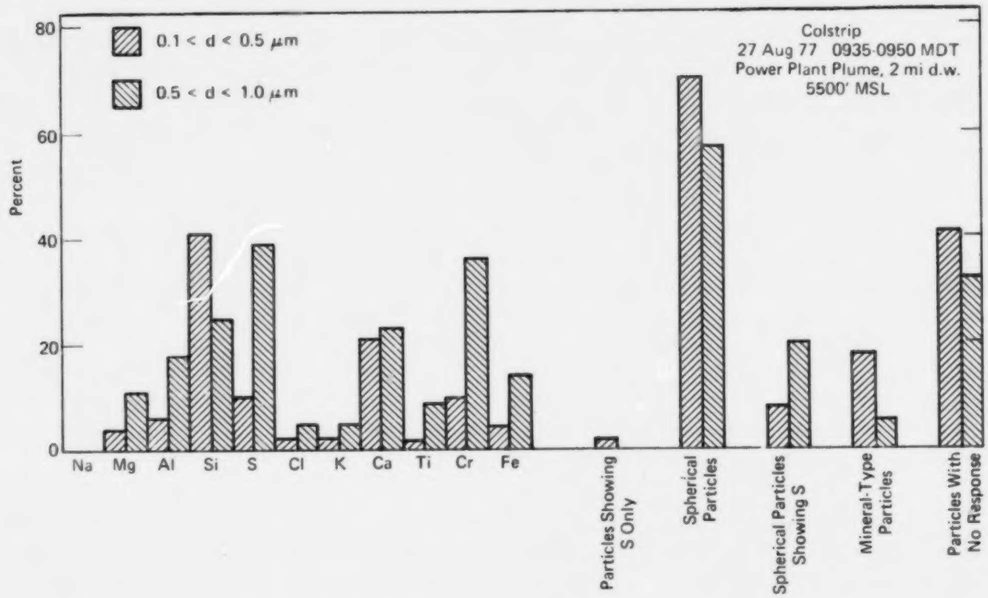

Figure 2.1a. SEM-EDX analysis of particles collected on Nuclepore filters and classified according to size, for 27 August 1977, 0935-0950 MDT.

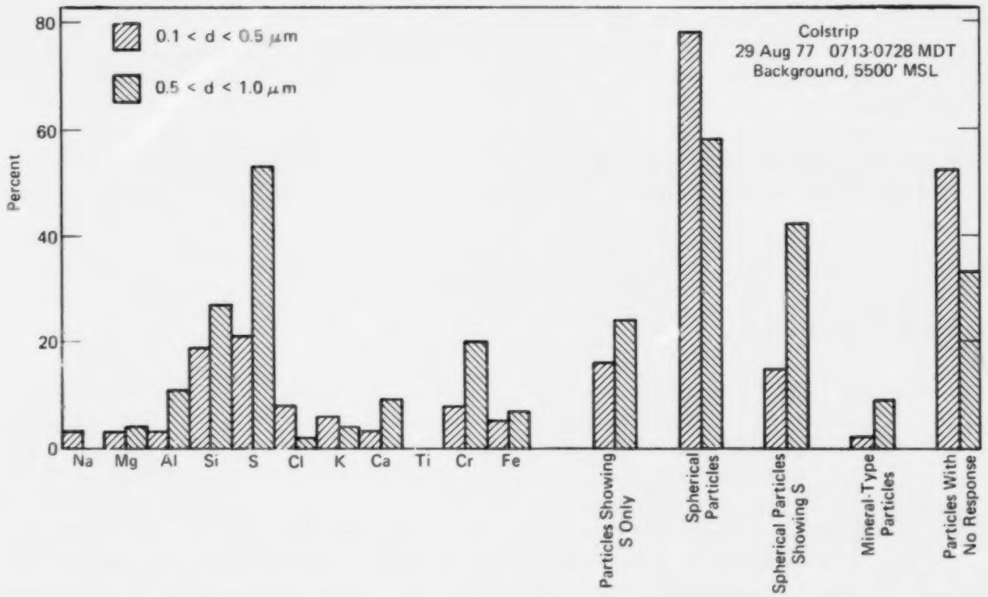

Figure 2.1b. SEM-EDX analysis of particles collected on Nuclepore filters and classified according to size, for 29 August 1977, 0713-0728 MDT.

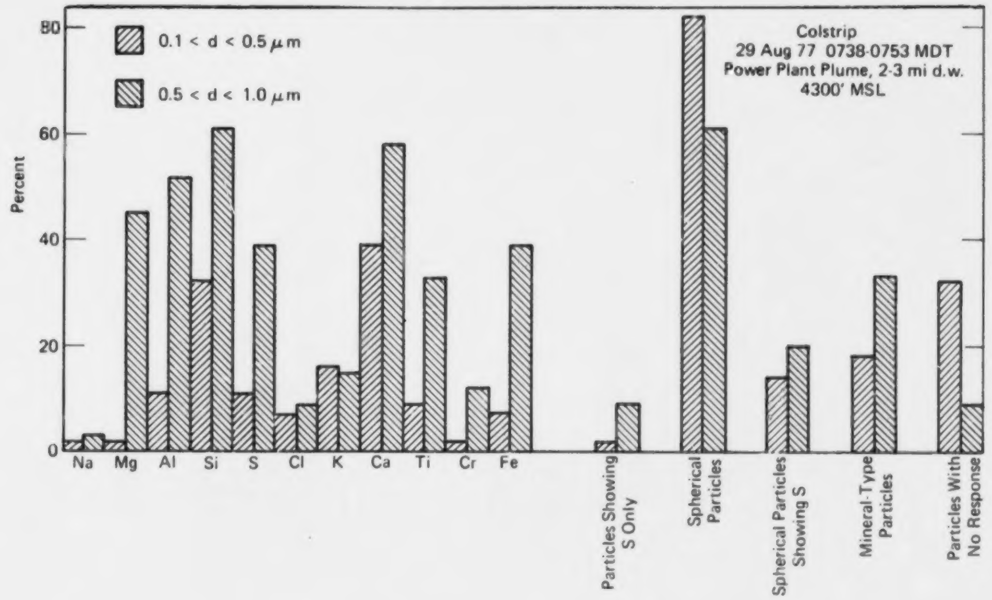

Figure 2.1c. SEM-EDX analysis of particles collected on Nuclepore filters and classified according to size, for 29 August 1977, 0738-0753 MDT.

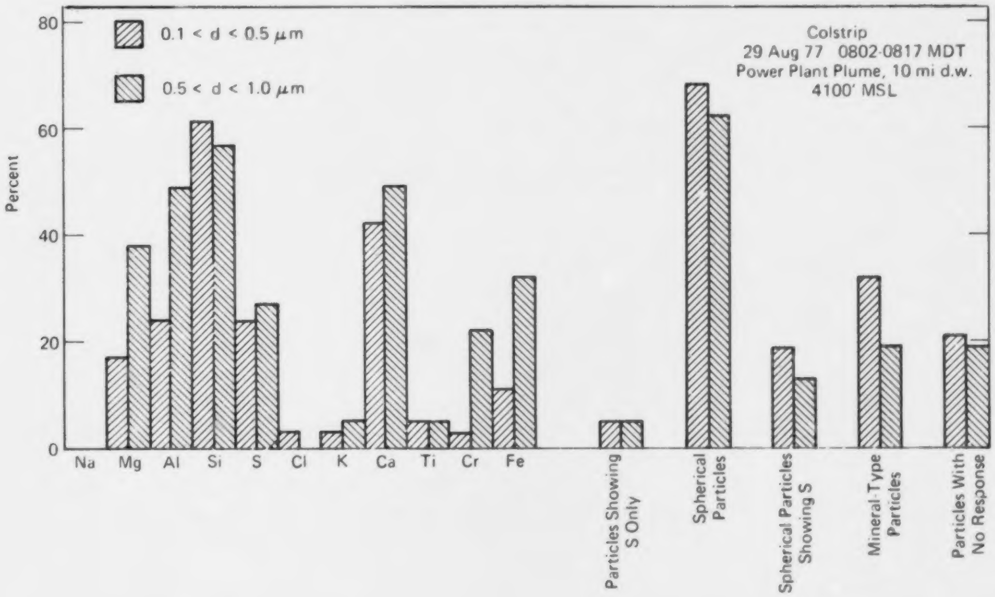

Figure 2.1d. SEM-EDX analysis of particles collected on Nuclepore filters and classified according to size, for 29 August 1977, 0802-0817 MDT.

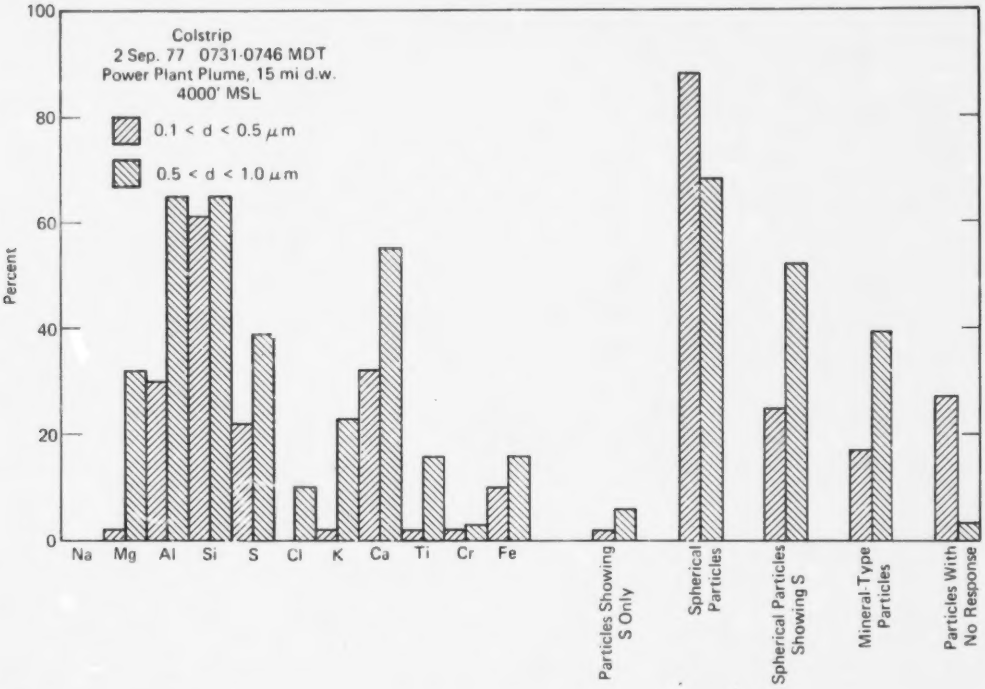

Figure 2.1e. SEM-EDX analysis of particles collected on Nuclepore filters and classified according to size, for 2 September 1977, 0731-0746 MDT.

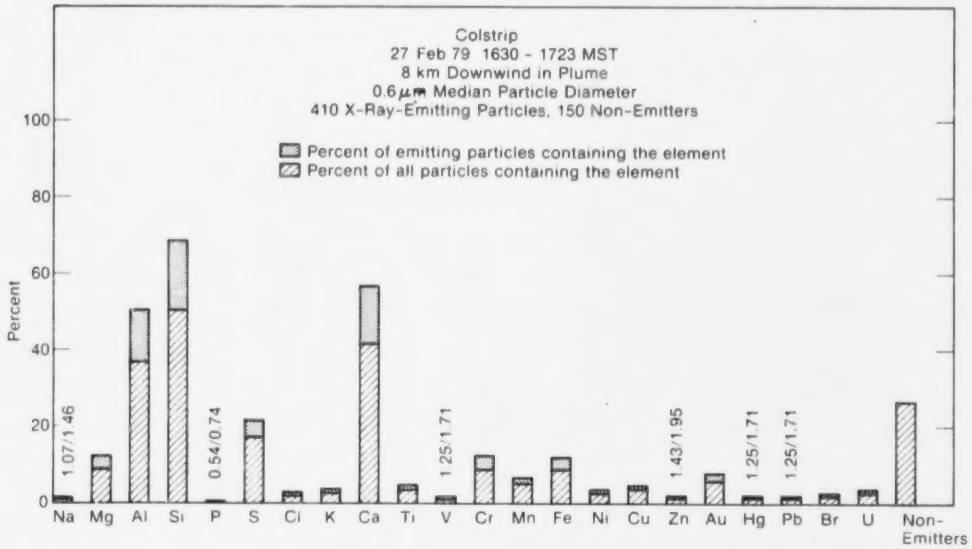

Figure 2.2a. SEM-EDX analysis of particles collected on Nuclepore filters, 27 February 1979, 1630-1723 MST. Numbers appearing above the very short bars indicate the percent of particles containing the element.

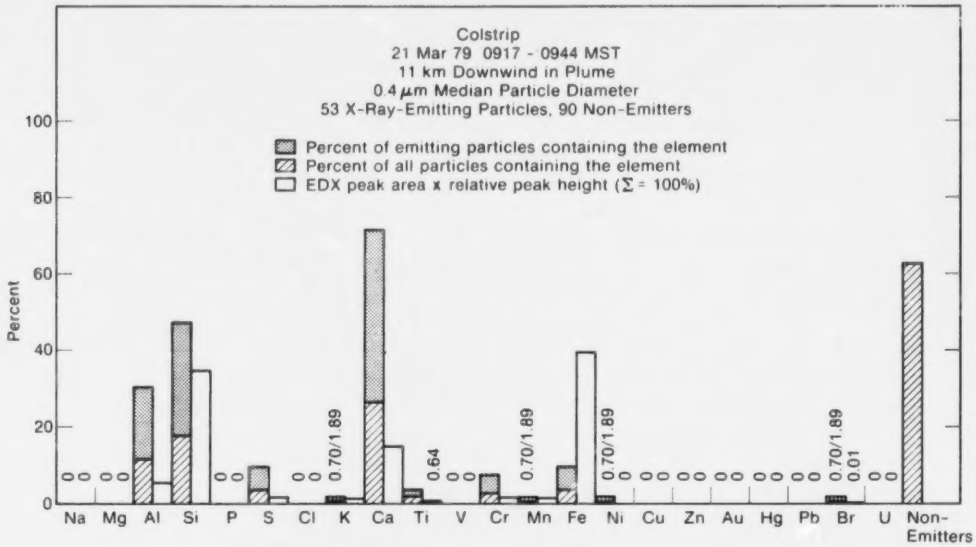

Figure 2.2b. SEM-EDX analysis of particles collected on Nuclepore filters, 21 March 1979, 0917-0944 MST. EDX peak area times relative peak height indicates emission strength and thus indicates the relative concentration of the element in the sample.

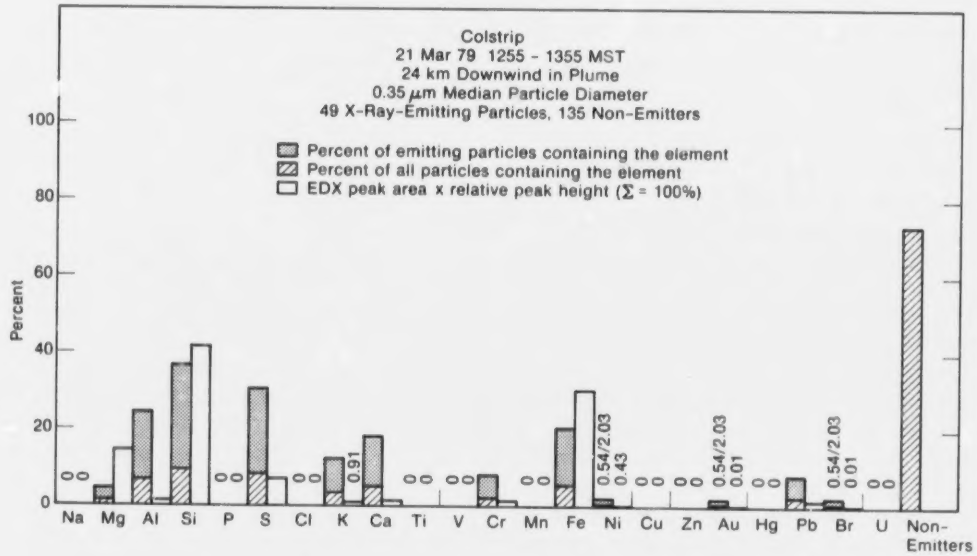

Figure 2.2c. SEM-EDX analysis of particles collected on Nuclepore filters, 21 March 1979, 1255-1355 MST. The numbers above very short bars indicate the percent of particles containing the element. EDX peak area times relative peak height indicates emission strength and thus indicates relative concentration of the element in the sample.

emissions account for 39.3 percent of total emissions. We see that Ca is rather widely distributed in the plume particles at this distance from the stacks, but nevertheless is apparently only a minor constituent, whereas Fe occurs in a few particles, but these few particles must be large and/or composed primarily of Fe.

In all of the examples except Figure 2.1b, which is a background sample, the elemental composition is like fly ash; Ca is prominent in all plume samples, and more so at the shorter downwind distances, as one would expect from this lime-scrubbed plume. Cr and Fe are prominent in the August-September 1977 samples, but much less so in the other samples. We think such variations in the metallic elements may be due to differences in coal or soil composition. Twelve samples collected during 1977 and 1979 were analyzed for the 22 elements shown in Figures 2.2a-c; of these, eight contained U in trace concentrations. All samples consisted of >50 percent spherical particles; plume and background samples were the same in this respect. While the spherical particles in the plume are mostly fly ash, those in background samples from other geographic areas are primarily organic or are nitrates and therefore non-emitting in the EDX analysis (containing no element heavier than F). Although particles with no EDX response (i.e., the non-emitters) represented 20 percent to 60 percent of all particles in the examples of Figures 2.1 and 2.2, analysis of other samples from these time periods has indicated an even wider variation from nearly zero to 75 percent or more. For two reasons the reported incidence of non-emitting particles must be considered only an approximation. First, the existence of electronic noise in the instrument requires that an elemental emission exceed the noise level by a predetermined factor. Second, compounds with an appreciable vapor pressure, such as organic substances, sulfuric acid, or to a lesser extent, the ammonium salts of sulfuric acid, sometimes evaporate in the vacuum of the SEM, especially under the heat of the electron beam. This also brings into question the accuracy of the analysis for sulfur-containing particles. We show that large numbers of very small sulfate particles exist in the plume. Obviously, the SEM-EDX analysis for sulfur-containing particles should be regarded only as a lower limit. The particles showing only sulfur are made up of a compound in which S is combined with a cation that emits X-rays that are not detectable in our analyzer. Likely cation candidates are ammonium ions and the particles are assumed to be ammonium sulfate or ammonium bisulfate.

In addition to the Nuclepore filter samples analyzed with the SEM-EDX, samples of plume aerosol particles of diameter 0.2 to 2.0 μm were collected on electron microscope screens mounted in front of the impactor jet nozzles of a four-stage Casella cascade impactor carried on the research aircraft. The screens were vacuum coated with $BaCl_2$ or with nitron (1,4-diphenyl-3,5-endoanilino-4,5-dihydro-1,2,4-triazole) for analysis of the sample for the presence of sulfate (Mamane and Pueschel, 1979a; Mamane and dePena, 1978) or of nitrate (Bigg *et al.*, 1974; Mamane and Pueschel, 1979b) with the transmission electron microscope (TEM). Figure 2.3a shows photomicrographs of particles collected on a $BaCl_2$-coated grid that was mounted in the fourth (final) stage of the cascade impactor. The network of small, elongated particles of uniform size and distribution is the $BaCl_2$ substrate. The more or less circular spots of a larger size than the $BaCl_2$ are the reaction product from the $BaCl_2$ and the sulfate particles that impacted on the grid. When

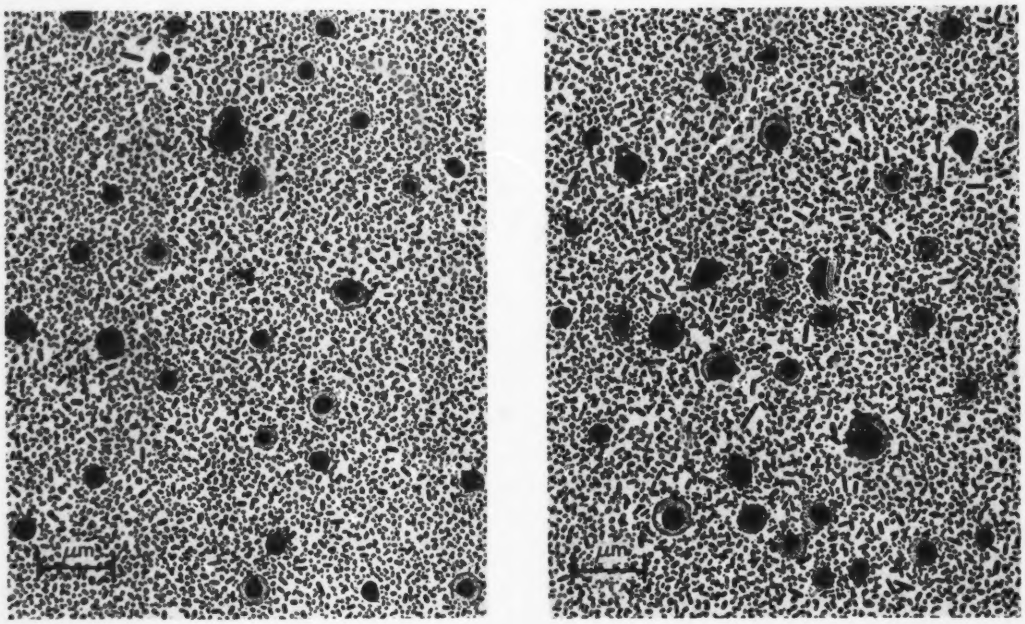

Figure 2.3a. TEM photomicrographs of particles collected on the fourth stage of a Casella cascade impactor. Elongated particles of uniform dispersion are the $BaCl_2$ coating; round spots indicate sulfate particles that have reacted with the $BaCl_2$, and are about double the diameter of the original particle. Left photomicrograph includes several particles that include mineral (or fly ash) centers.

viewed in the TEM, only four of the darkest spots in the left-hand photomicrograph have opaque centers that are fly ash particles. The diameters of the $BaSO_4$ reaction spots are approximately twice those of the original sulfate particles; the smallest particles that appear in these photomicrographs were therefore about 0.2 µm initial diameter.

Figure 2.3b shows photomicrographs of particles collected on grids that were coated with nitron before (right) or after (left) collection of the sample. The nitron method is a specific test for nitrate by the formation of needle-like crystals or masses of needles. There are no nitrate-containing particles in the left-hand photomicrograph, and only two in the right that may contain nitrate. These impactor samples from power plant plumes do not show appreciable numbers of nitrate particles on the fourth stage impactor grids, but do frequently show nitrate in the next larger (third stage) size fraction, i.e., most of the nitrate is found in particles of 1 µm diameter and larger, and there are few nitrate-containing particles smaller than 1 µm (Van Valin *et al.*, 1980). This finding is consistent with those of other studies (Parungo *et al.*, 1980; Pueschel *et al.*, 1979a). The explanation is that nitrate and

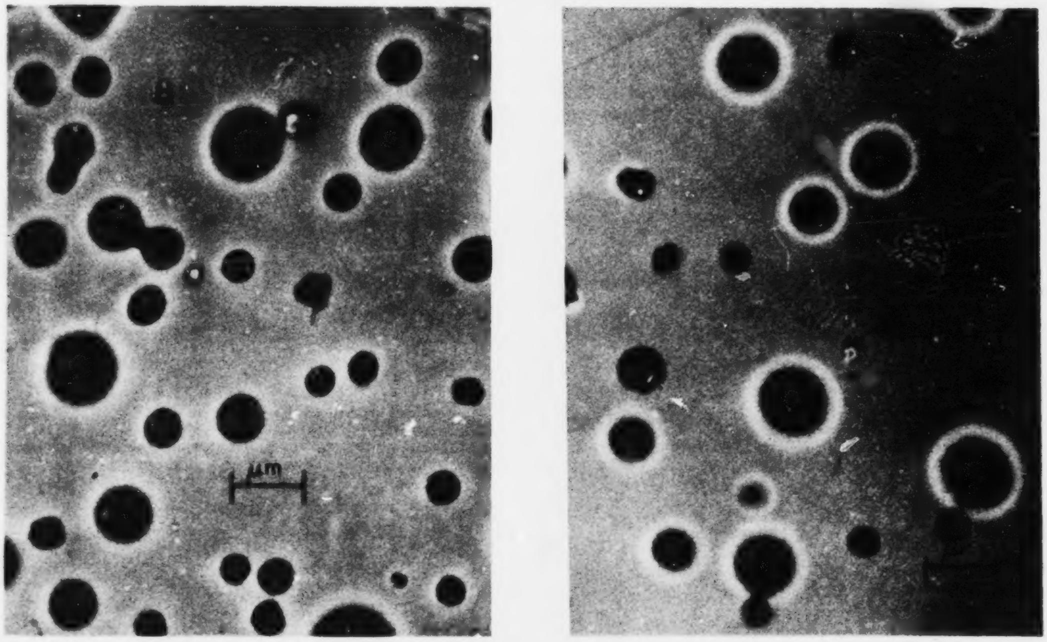

Figure 2.3b. TEM photomicrographs. Left: particles collected on an impactor grid that was coated with nitron following collection. Right: grid was coated with nitron before sample collection. Spots with clear halos that shade into a darker center represent sulfates and are about three times the diameter of the particle they represent.

nitric acid particles are very hygroscopic and grow to above-micron-sized droplets rapidly at slight supersaturations or even subsaturation. The atmospheric residence times of these particles is limited by rapid fallout and washout. The examples in Fig. 2.3b are typical of fourth-stage nitron-coated samples, however; the spots with the clear halos and increasing density toward the centers are typical of (though not specific for) sulfates, and the spots with neither halos nor shading are probably fly ash. Fly ash or other mineral particles with sulfate coatings would show as uniformly dense, sharp-edged spots surrounded by the clear halo. In the left-hand photomicrograph there are two particles that may be mineral, with very faint halos; most of the rest are probably sulfates. The right-hand example includes eight spots that are probably fly ash particles of ~0.5 μm diameter without the sulfate coating. The diameters of the spots produced by sulfate on nitron are about three times those of the original particles; thus, the sulfate particles in these two nitron-coated samples were from 0.2 to 0.6 μm diameter, approximately.

Ice Nuclei

Coal contains a variety of impurities that, if occurring as constituents of aerosol particles, may be capable of acting as ice nuclei (IN). Because of the importance of the ice phase in the formation of precipitation, the potential for inadvertent weather modification as a consequence of increasing energy production in the western states has been of concern to atmospheric scientists. However, research has given an ambiguous picture of the impact of power plant effluents on the atmospheric ice nucleus budget. Schnell *et al.* (1976) and Pueschel *et al.* (1979b) found no difference in the number density of IN between samples from the plumes of the Four Corners and Homer City, Pennsylvania, power plants and samples of the surrounding clean air. Parungo *et al.* (1978a) found similar results when samples were handled in a manner similar to that described by Schnell *et al.*; however, Parungo *et al.* discovered that IN could be activated in the plume samples by subjecting the sample to high water supersaturation, high temperature, or high vacuum, or combinations of these treatments. Research in our laboratory suggests that tests using different modes of nucleation will produce different results, i.e., the filter method used by Schnell *et al.* and Parungo *et al.* depends upon the deposition (reverse of sublimation) or condensation-followed-by-freezing mechanisms, while the acoustic counter method depends upon the contact nucleation mechanism (Langer *et al.*, 1967). Because of its ease of sample collection, the filter method of Bigg *et al.* (1963), as modified to use Nuclepore filters and a static water vapor source, was used exclusively for the airborne measurements at Colstrip, and for the majority of samples at the mobile laboratory at Hay Coulee. The acoustic counter at Hay Coulee was used to the extent possible. The first report on NOAA/ERL activity at Hay Coulee (Abshire *et al.*, 1978) presented ice nucleus measurements by both methods, showing equivalent concentrations of IN in the atmosphere prior to power plant operation. The second report (Van Valin *et al.*, 1979) showed that the ice nucleus concentration, as measured by the acoustic counter method during a well-documented plume exposure at Hay Coulee (23 August 1977, 0828-0854 MDT), was raised by nearly an order of magnitude. Similar episodes of plume exposure at Hay Coulee, while not always as striking, produced similar increases in the IN-acoustic counter method. During times of non-exposure to the plume this method showed IN concentrations in the range 1-4 ℓ^{-1}, which is entirely consistent with the levels reported earlier. However, filter-method samples collected during these times of plume exposure were not different from those collected during non-exposure times. Hay Coulee filter-method ice nucleus measurements for the four field periods in 1976 and 1977 are listed in Table 2.1. In addition to the comparison of IN concentrations between plume and non-plume exposures, the samples were segregated by time of collection (morning, mid-day, and afternoon), but the variability is so large that one cannot have confidence in a possible pattern. In like manner, the plume and ambient atmosphere samples cannot be shown to be different; the present methods are not sufficient to identify the plume as a source of IN. The filter-method (modified from Bigg *et al.*, 1963) IN measurements of samples collected with the aircraft are presented in Table 2.2. Again, no difference can be shown between the plume and ambient IN concentrations.

The indication that IN were not generated during operation of coal-fired power plants has caused some puzzlement and skepticism. The measurement of very much higher IN concentrations with the acoustic counter during plume exposure periods and the observance of enhanced snowfall downwind from a power plant (Parungo *et al.*, 1978b) lends weight to the belief that the filter method used in this study is adversely affected by the surface absorption of substances, probably soluble sulfates, that deactivate the deposition and condensation-followed-by-freezing modes of nucleation. The contact mode of nucleation is therefore affected to a significantly smaller degree. Further research on the ice-nucleating characteristics of plume aerosols continues.

TABLE 2.1. ICE NUCLEUS CONCENTRATIONS (ℓ^{-1}) AT HAY COULEE AS MEASURED BY THE FILTER METHOD, EXPRESSED AS MEANS AND ONE-SIGMA STANDARD DEVIATIONS

Period	All samples	Before 1000 MDT	1000-1300 MDT	After 1300 MDT
May-June 76	(n=34) 2.03±1.63	(n=9) 3.67±1.42	(n=13) 1.80±1.64	(n=12) 1.07±0.58
Aug-Sept 76	(n=45) 2.78±1.15	(n=17) 3.12±1.15	(n=17) 2.54±1.27	(n=11) 2.62±0.91
May-June 77	(n=44) 2.43±1.56	(n=22) 2.83±1.53	(n=11) 2.58±1.89	(n=11) 1.54±0.75
Aug-Sept 77	(n=44) 6.43±3.59	(n=15) 7.74±5.00	(n=11) 5.87±3.08	(n=18) 5.68±2.02

Period	In Plume (all times)	Ambient (all times)
May-June 76	---	---
Aug-Sept 76	---	---
May-June 77	(n =9) 3.21±2.16	(n=35) 2.24±1.32
Aug-Sept 77	(n=10) 4.64±2.54	(n=34) 6.96±3.71

Note: n is the number of samples.

Plume Dispersion

Plume dispersion rates determine the volume of the atmosphere in which compositional changes take place and the land area that is affected by deposition of pollutants. Cross-sectional characteristics, e.g., concentration profiles of aerosols and gases at various distances downwind, are used to measure plume dispersion. We present here several examples of plume profiles. Figures 2.4 and 2.5 show the Aitken nucleus (AN) concentrations found in the horizontal and vertical profiles on 1 September 77. Figures 2.6 and 2.7 are similar profiles done on 31 August 77. Figures 2.8 and 2.9 are representations of the maximum change from background of the other constituent concentrations measured at the same time. Figures 2.10 and 2.11 are profiles of the power plant plume representing AN and ozone concentrations, respectively, as measured 25 km downwind from the power plant on 16 February 1977. The inset in the upper left-hand corner of Figure 2.10 represents the plume dimensions 0.5 km downwind from the stacks, according to Turner (1970); the symbols on the x-axis 10 m on either side of the centerline indicate stack spacing and diameter. Figure 2.12 shows the ozone profile a fraction of an hour later and at a downwind distance of 8 km. Figure 2.12 represents only the upper part of the plume following a reduction of wind velocity from approximately 13 m sec^{-1} to 4 m sec^{-1} and a decrease in atmospheric stability resulting in either plume looping or fumigation.

TABLE 2.2. ICE NUCLEUS CONCENTRATIONS (ℓ^{-1}) FROM AIRBORNE SAMPLE COLLECTION AS MEASURED BY THE FILTER METHOD, EXPRESSED AS MEANS AND ONE-SIGMA STANDARD DEVIATIONS.

Period	In Plume	Ambient
May-June 76		(n=14) 0.88±0.60
Aug-Sept 76	(n=3) Ave = 2.56	(n=38) 1.19±1.02
Feb 77	(n=5) 1.36±0.31	(n=5) 0.93±0.34
May-June 77	(n=7) 1.58±0.68	(n=48) 1.61±1.29
21-25 Aug 77	(n=2) Ave = 3.04	(n=19) 2.07±1.96
27 Aug-2 Sept 77	(n=14) 4.56±4.06	(n=7) 7.30±5.26
Aug-Sept 77 (all flights)	(n=16) 4.37±3.82	(n=26) 3.48±3.87

Note: n is the number of samples.

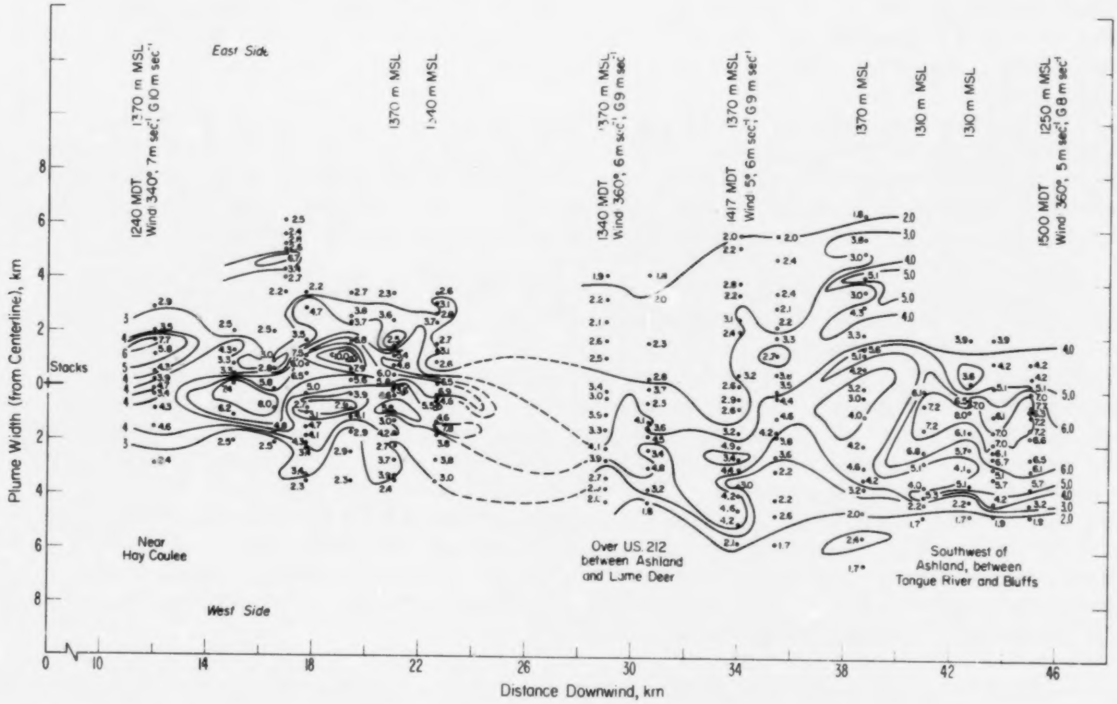

Figure 2.4. Horizontal profile, based on AN measurements, of the Colstrip power plant plume from 12 to 45 km downwind on 1 September 77, 1240-1500 MDT.

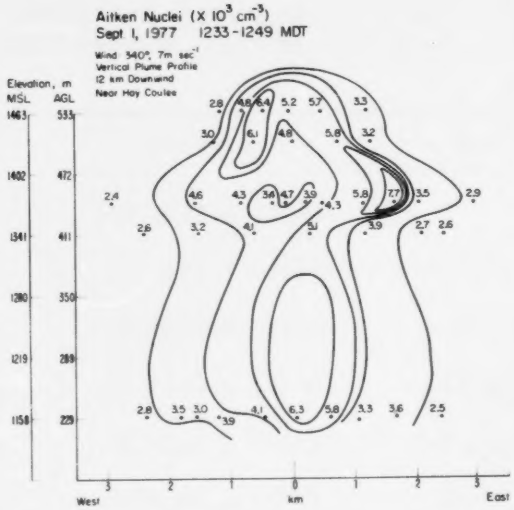

Figure 2.5. Vertical profile, based on AN measurements, of the Colstrip power plant plume on 1 September 1977, 1233-1249 MDT.

These three sets of plume measurements demonstrate the inhomogeneities along the x, y, and z axes that are typical for coal-fired power plant plumes. These findings are in contrast to theoretical treatments of plume dispersion that predict Gaussian distributions of plume properties along both the z and y axes (e.g., Turner, 1970).

A similar discrepancy is found if one compares the measured with the predicted plume cross sections. Table 2.3 compares the measured and predicted plume dimensions (Turner, 1970) at distances from the stacks of ½, 8 and 25 km for stability classes D, C, and D respectively. It follows from Table 2.3 that theory overestimates plume height z, but underestimates the plume width.

This discrepancy follows from at least three factors: (a) measurements with the mobile laboratory at Hay Coulee show, on the very short term, wind direction commonly varying over a range of 40 to 70 degrees, (b) terrain factors in the vicinity of Colstrip contribute additional deflection (see Influence of topography on plume transport), (c) Aitken particles and ozone are far from ideal as plume tracers, especially because the profile dimensions and measured constituent concentrations indicate a reactive mixture involving a particle-generating process. The third conclusion follows from Figures 2.4 and 2.6, which show that the AN concentrations at 40-50 km downwind from the stacks are equal, or nearly so, to those found 12 km downwind, in spite of an observed dispersion of the plume. These findings conclusively show, however, that the plume does not follow a Gaussian distribution, which is the basis for all theoretical plume dispersion models.

Additional examples of plume dimensions and constituent concentrations follow. On 30 August 1977 a vertical plume profile was obtained near Lame Deer, Montana, which is 29 km on a straight line from Colstrip (Figure 2.13). The plume's external profile and underlying topography are shown in Figure 2.14. The shape of the profile and the reasons for the plume's location near Lame Deer are discussed below. Figure 2.15 summarizes the plume constituent

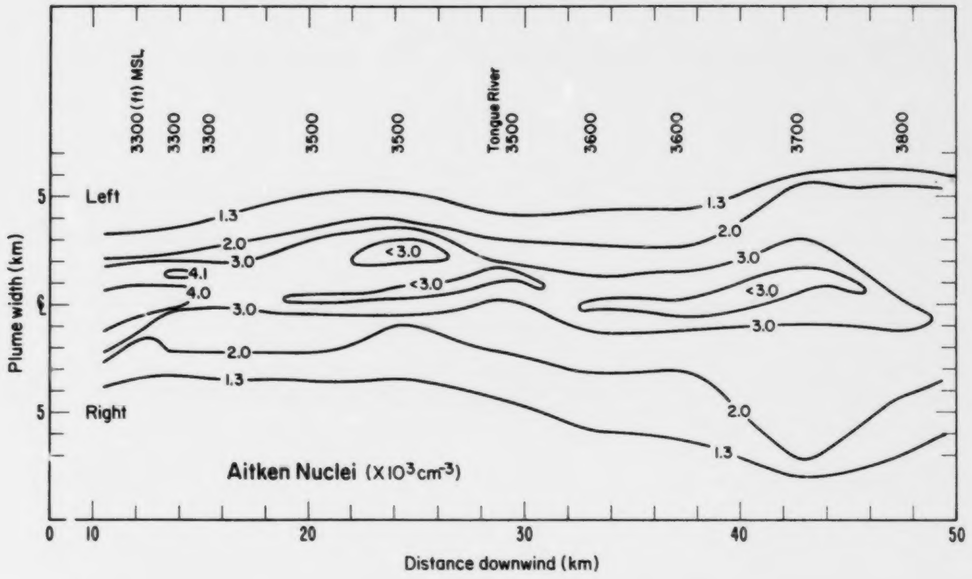

Figure 2.6. Horizontal profile, based on AN measurements, of the Colstrip plume on 31 August 1977, 1024-1140 MDT.

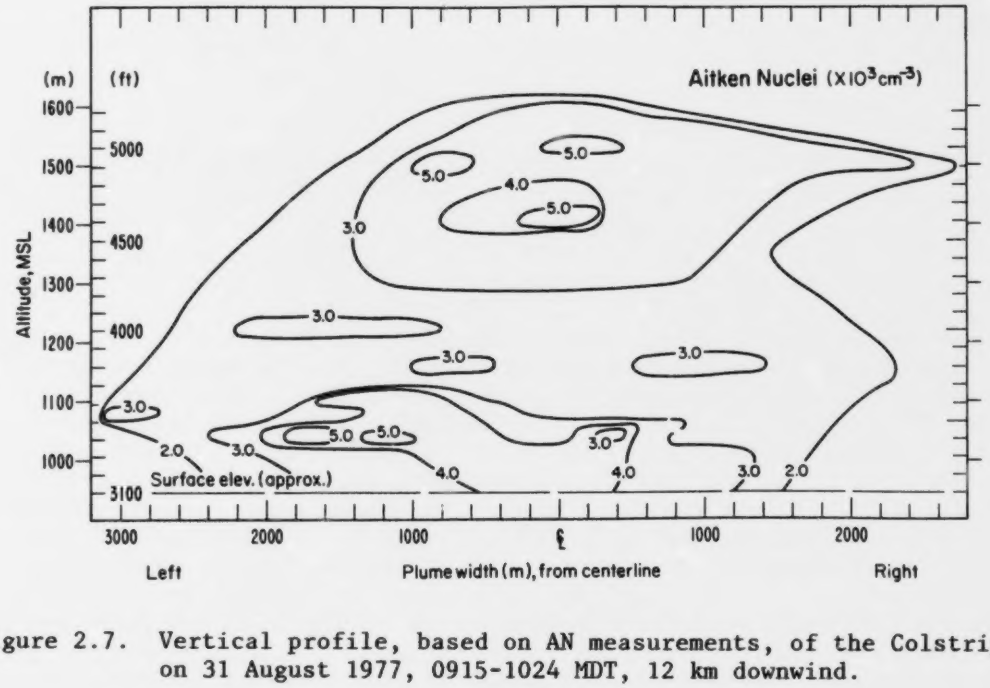

Figure 2.7. Vertical profile, based on AN measurements, of the Colstrip plume on 31 August 1977, 0915-1024 MDT, 12 km downwind.

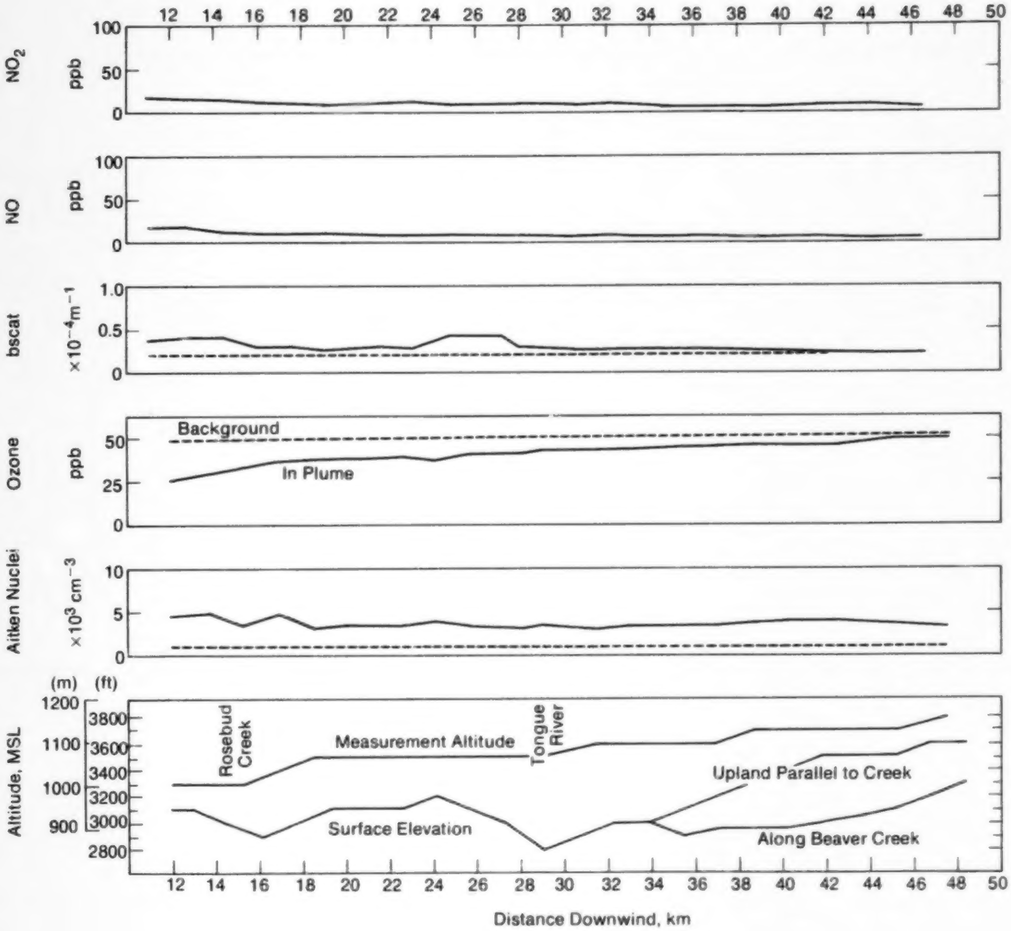

Figure 2.8. Maximum deviation of plume constituent concentrations from background on 31 August 1977, 1024-1140 MDT.

concentrations at the various levels in the plume. This part of the plume was at least 5 hours removed from the power plant; the transport had occurred during the night, with a temperature of about 11°C and relative humidity exceeding 80 percent. In spite of the time lapse, the NO had been only partially oxidized to NO_2, and O_3 was essentially absent in the center of the plume. The AN concentration is also rather low, considering the time of possible reaction, as well as the residual SO_2 concentration. The evidence here is rather persuasive that solar energy input has a strong effect upon the rates, or even the occurrence, of reactions of the constituent substances.

Figure 2.16 shows the data obtained during a spiral descent from 3600 m at the rate of 60 m/min through the plume. Figure 2.17 is another plume profile at the same location, showing somewhat higher concentrations, but with

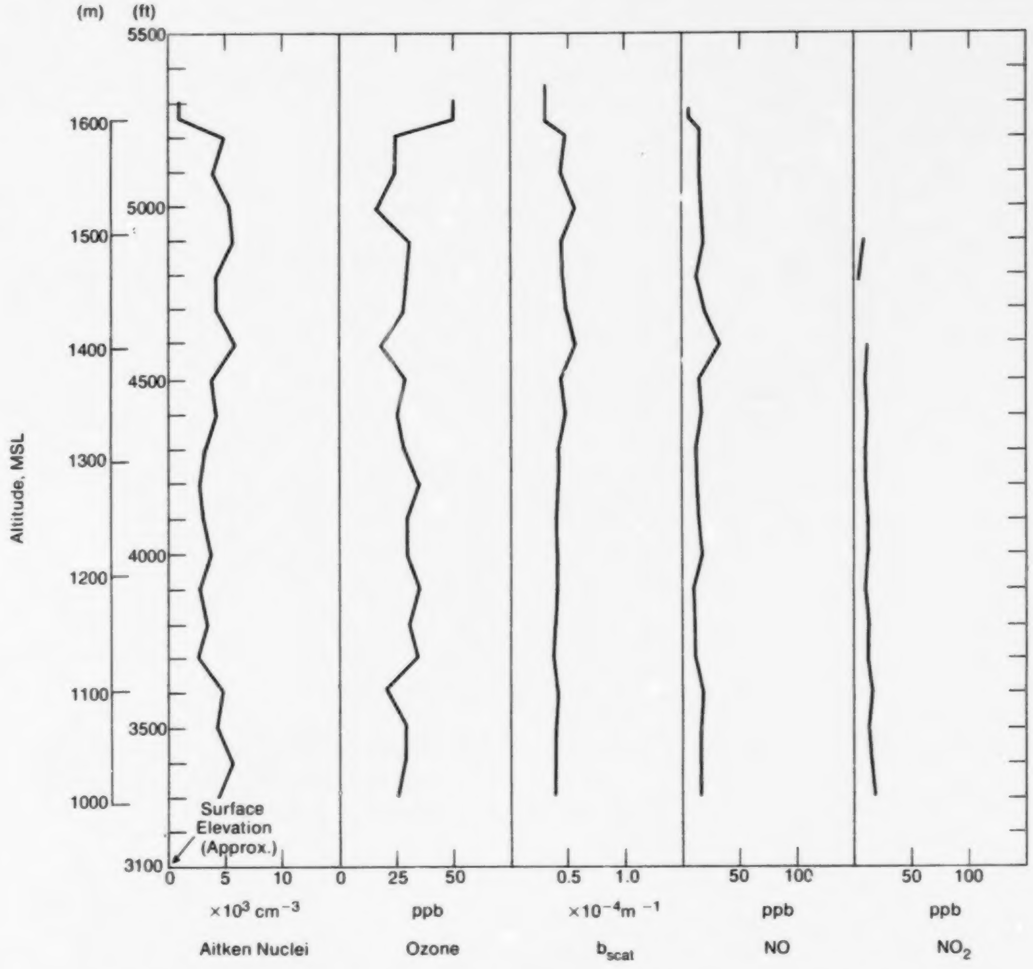

Figure 2.9. Maximum deviation of plume constituent concentrations from background during a vertical profile 12 km downwind, 31 August 1977, 0915-1024 MDT.

a consistent ratio in all cases. Figure 2.18 is the representation of plume vertical profile limits at the same time, as determined by the point at which the O_3 concentration was reduced from background by approximately double the instrument electronic noise level. Figure 2.19 and 2.20 are the corresponding constituent concentrations and profile obtained about 12 km downwind. The wind direction and velocity during this time period were SSW at 0-3 m sec^{-1} at plume altitude; stability index according to Turner (1970)could have been D or C, considering the isothermal layer at plume elevation. Table 2.4 lists the predicted and measured dimensions of the plume. Theory understates both plume width and thickness at the shorter distance, but overestimates both at the 12 km distance. Height estimates are rather uncertain and subject to extreme variation according to the atmospheric stability. The measured plume is thinner in overall vertical dimensions at 12 km than at 4 km; this appears

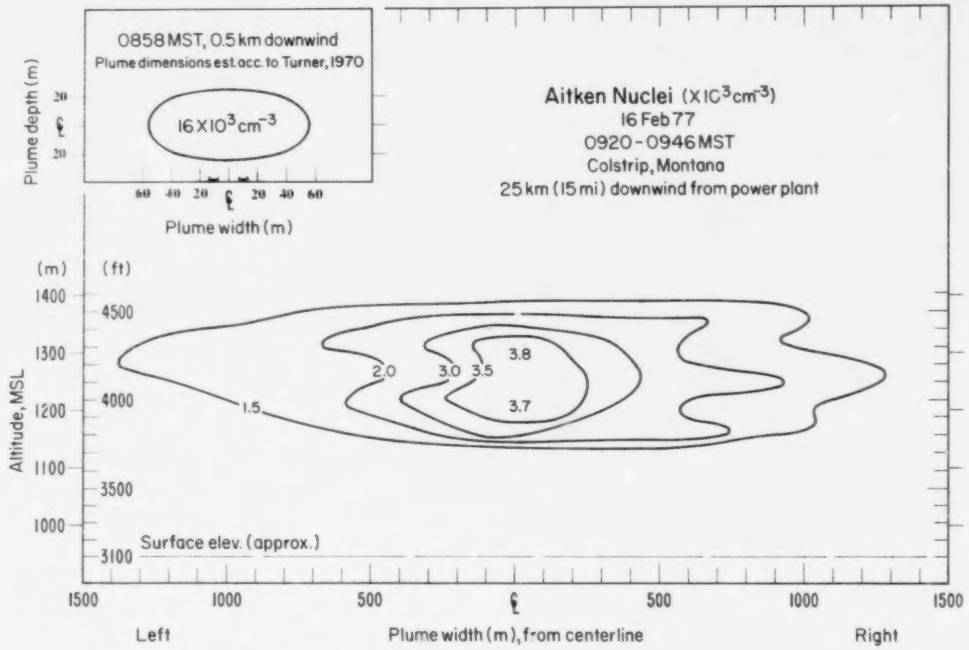

Figure 2.10. AN vertical contour profile of the Colstrip plume on 16 February 1977, 0920-0946 MST; the inset represents the theoretical plume boundary and measured maximum concentration of AN.

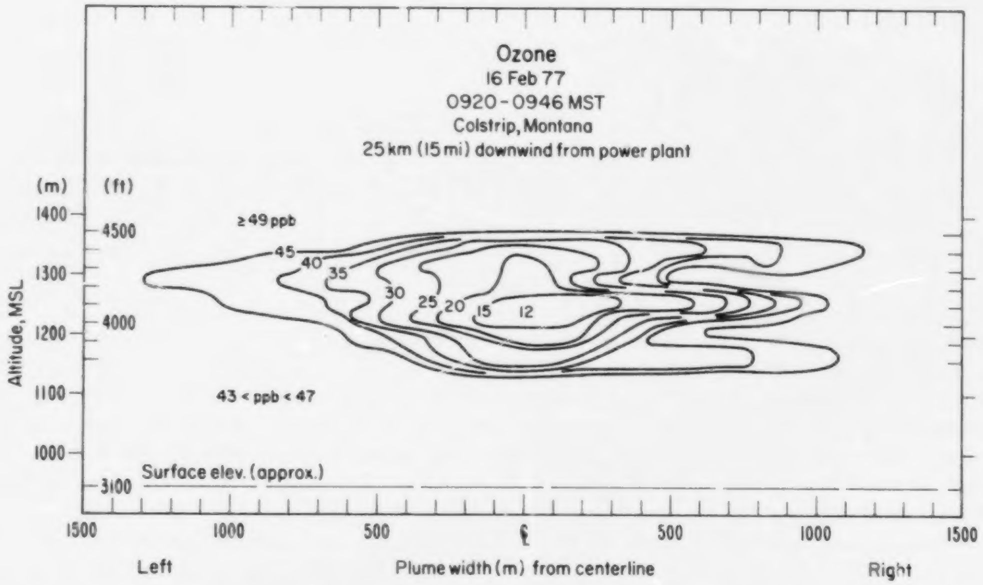

Figure 2.11. Ozone vertical contour profile of the Colstrip plume on 16 February 1977, 0920-0946 MST, corresponding to AN concentrations shown in Figure 2.10.

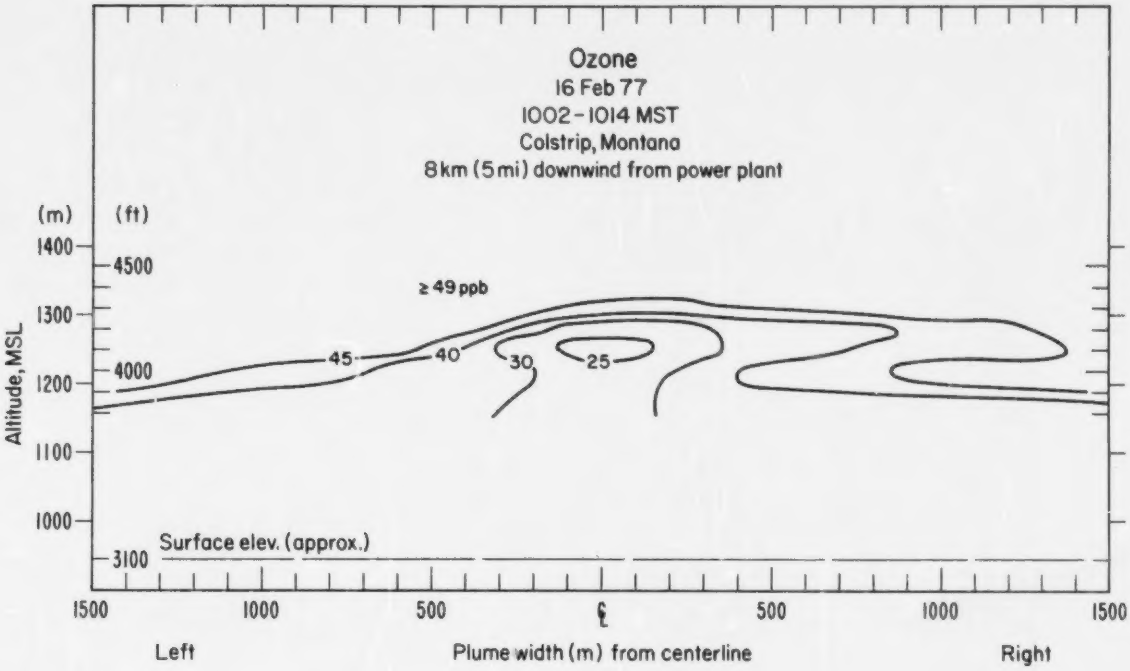

Figure 2.12. Ozone vertical contour profile of the Colstrip plume.

TABLE 2.3. MEASURED AND PREDICTED DIMENSIONS FOR THE COLSTRIP POWER PLANT PLUME ON 16 FEBRUARY 1977

Distance (km)	Width (m)		Height (m)	
	Measured	Predicted	Measured	Predicted
½ (Stability Class D)	--	100	--	40
8 (Stability Class C)	>1800	1400	135<z<350	800
25 (Stability Class D)	2800	2400	250	460

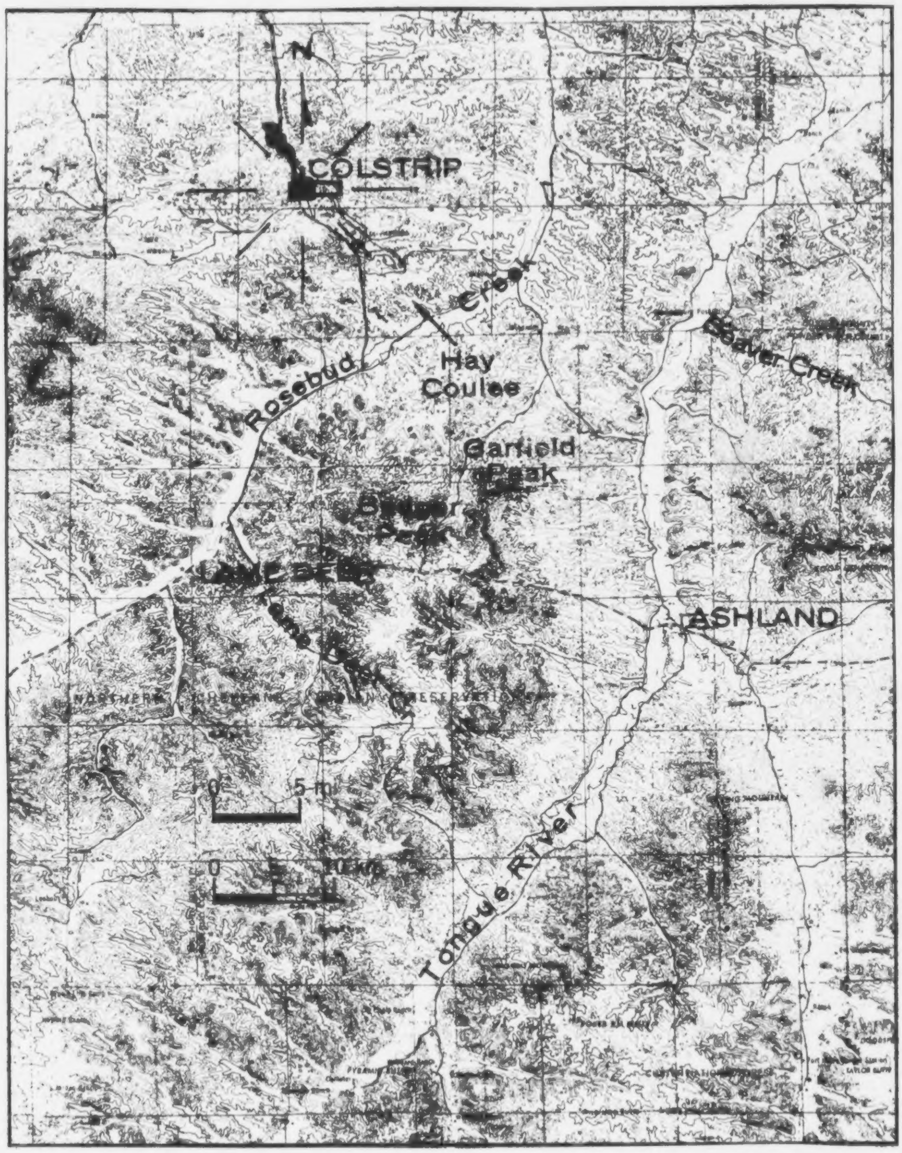
N
COLSTRIP
Rosebud Creek
Hay Coulee
Beaver Creek
Garfield Peak
LAME DEER
ASHLAND
NORTHERN CHEYENNE INDIAN RESERVATION
0 5 mi
0 5 10 km
Tongue River

Figure 2.13. Topographic map of the Colstrip area (U.S. Geological Survey).

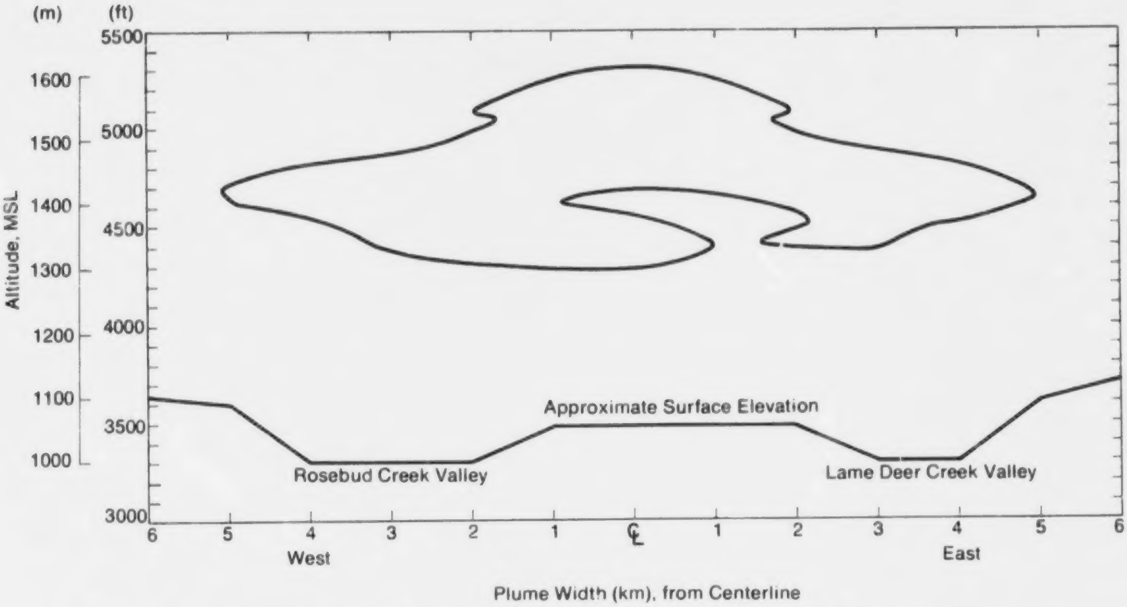

Figure 2.14. Vertical boundary profile of the Colstrip plume according to measured AN concentrations, 30 Aug. 1977, 0645-0735 MDT, 29 km downwind.

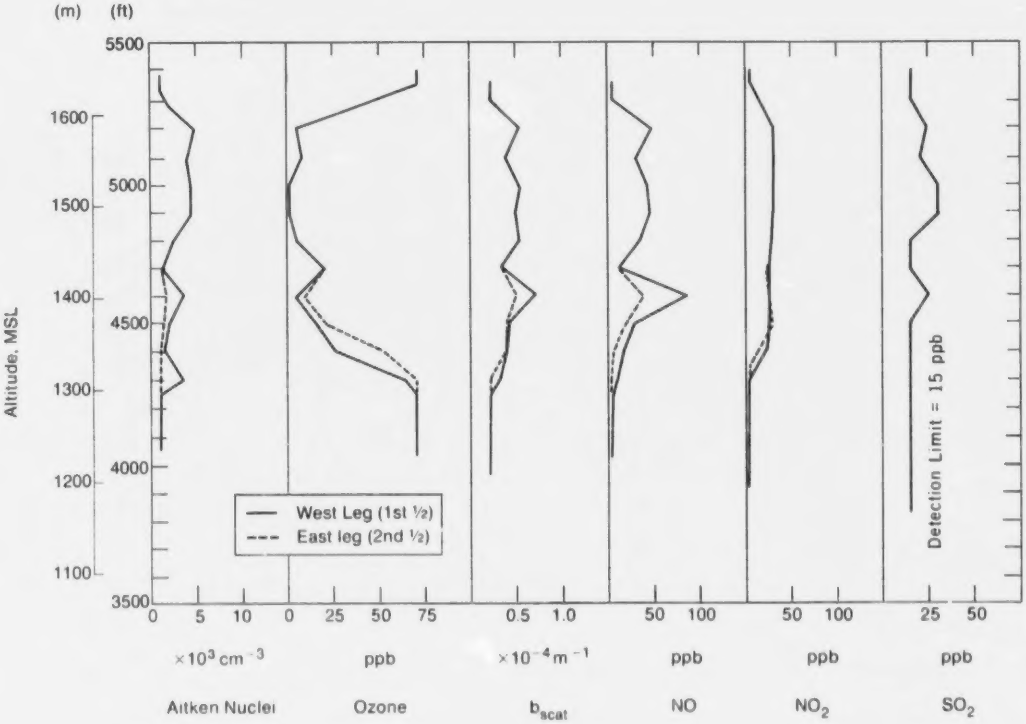

Figure 2.15. Maximum deviation of the plume constituent concentrations from background during the vertical profile of Figure 2.14.

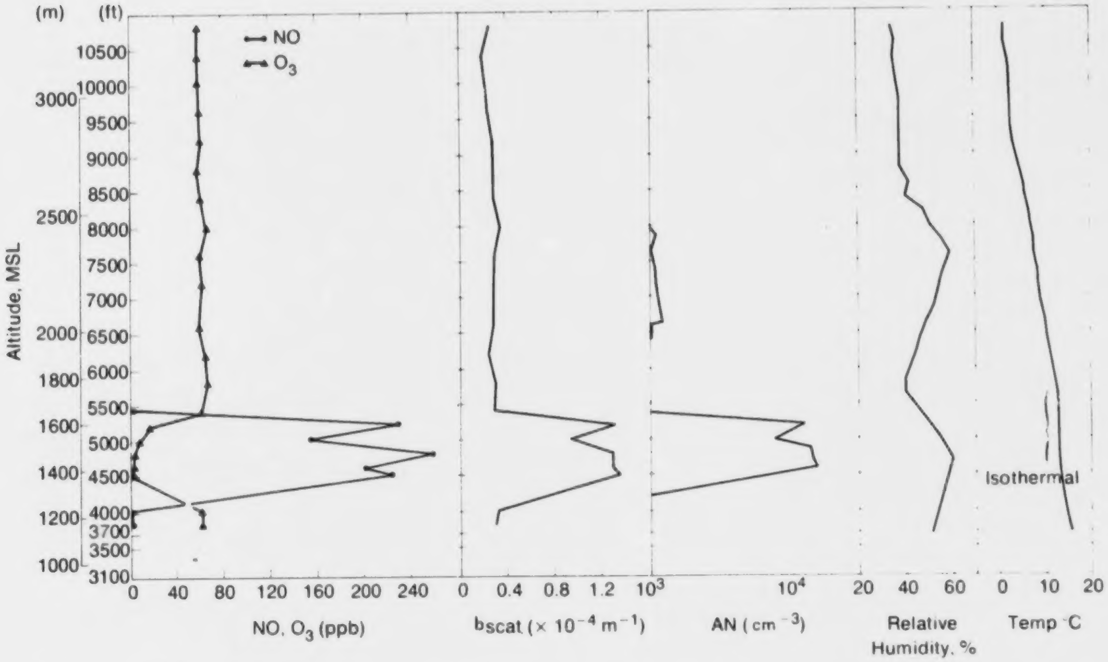

Figure 2.16. Constituent concentrations during a descending spiral from 3600 m MSL, 28 Aug. 1977, 0544-0621 MDT, 4 km downwind.

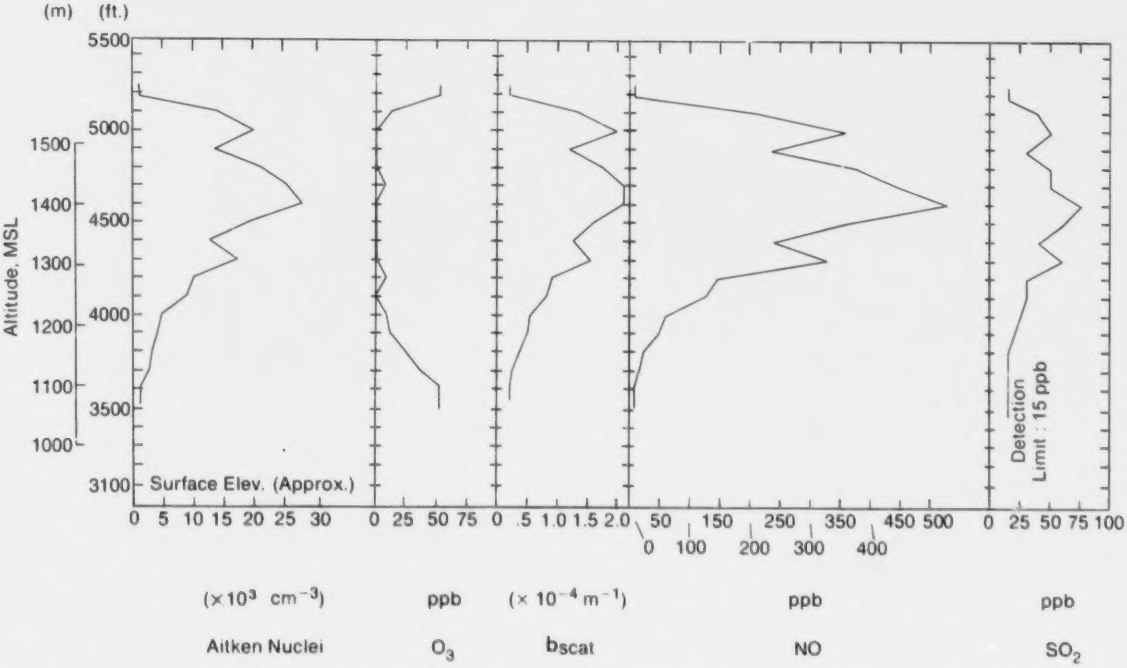

Figure 2.17. Maximum deviation of plume constituent concentrations from background during a vertical profile immediately following the profile of Figure 2.16 (0635-0723 MDT).

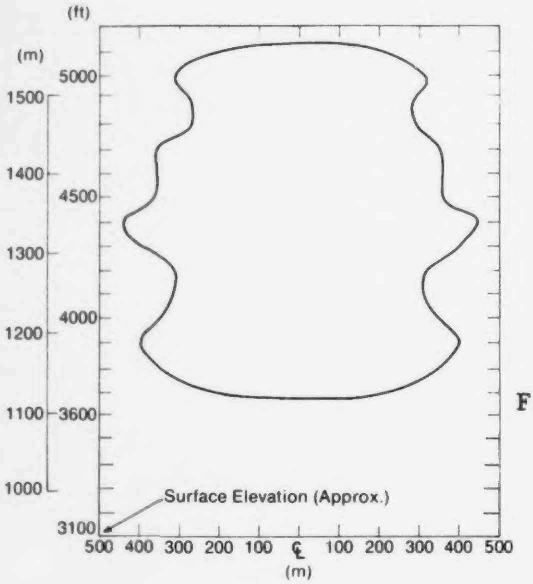

Figure 2.18. Vertical boundary profile of the Colstrip plume according to measured O_3 concentrations, 28 Aug. 1977, 0635-0723 MDT, 4 km downwind.

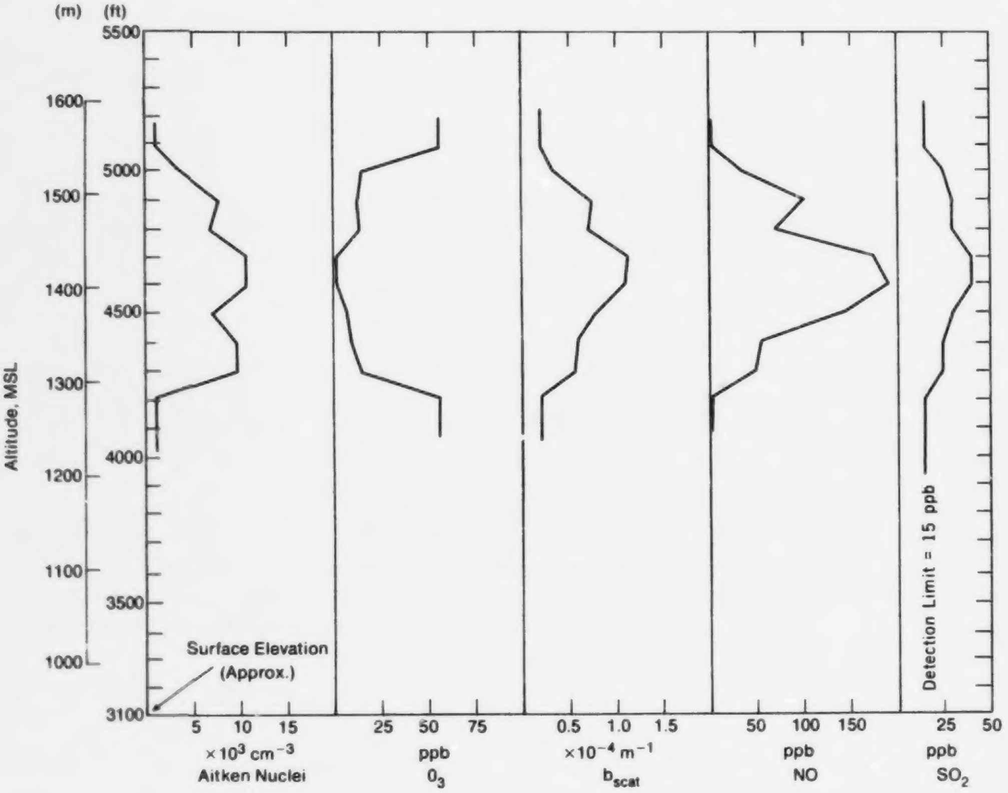

Figure 2.19. Maximum deviation of plume constituent concentrations from background during a vertical profile, 28 Aug. 1977, 0800-0834 MDT, 12 km downwind.

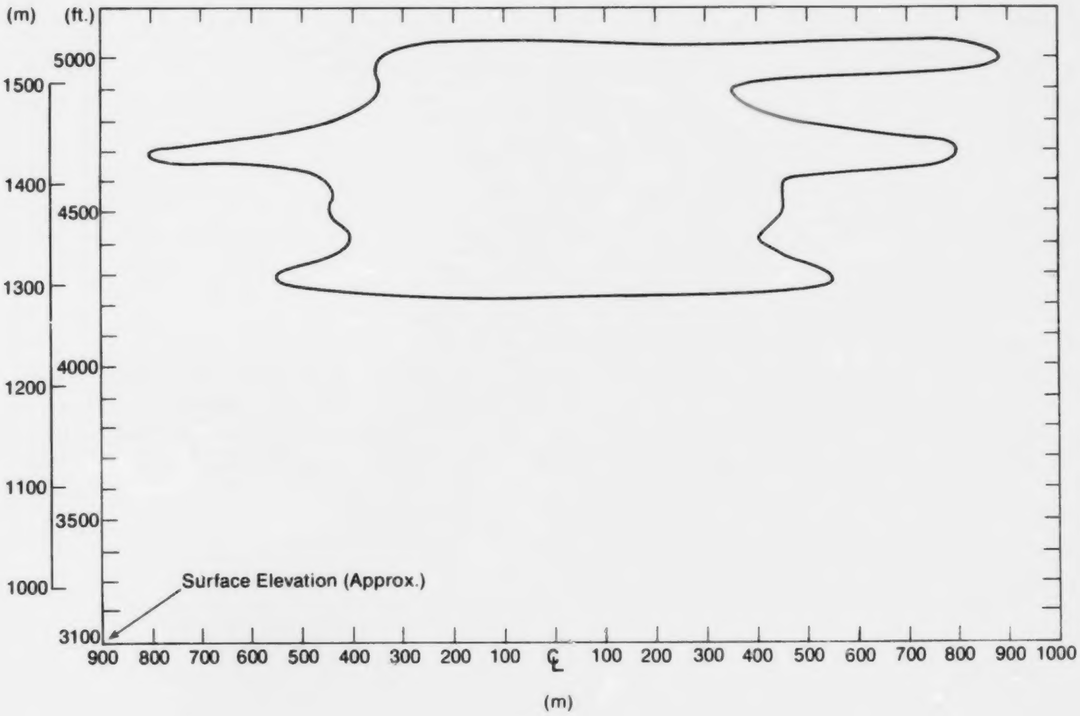

Figure 2.20. Vertical boundary profile of the Colstrip plume according to measured O_3 concentrations, 28 Aug. 1977, 0800-0834 MDT, 12 km downwind.

to be the consequence of a slight increase in wind velocity during the intervening time of roughly 1 hour. The plume at 12 km also shows evidence, on its top surface, of wind shear; our pibal sounding at Hay Coulee, as well as aviation weather reports, indicated winds above plume altitude of 270°-300°.

On 29 August 1977, at 5 km and 16 km downwind, repeated penetrations of the plume in its densest part were made by aircraft. The data are presented in Table 2.5. Repeated passes at the same location (16 km downwind) and altitude (1220 m MSL) permitted a look at the relationships among the gases O_3, NO_2, and NO. Figure 2.21 shows the concentrations of NO_2 and O_3 relative to NO. The two lines were obtained by least squares fit; the coefficients of determination (r^2), were 0.66 and 0.58, indicating rather modest fit. The background O_3 concentration of 68 ppb where NO and NO_2 were near zero was not included in the curve-fitting calculations. Both NO_2 and O_3 are higher in the less dense portions of the plume, representing both more dilution and a greater degree of reaction between O_3 and NO, but the O_3 decline is greatly in excess of that required to produce the small amount of NO_2 actually measured at least in consideration of the plume expansion as indicated by diminution of NO concentration. It is apparent that the reaction with NO is not the primary loss mechanism for O_3.

TABLE 2.4. MEASURED AND PREDICTED DIMENSIONS FOR THE COLSTRIP POWER PLANT PLUME ON 28 AUGUST 1977

Distance (km)	Width (m)			Height (m)		
	Measured	Predicted (stability index)		Measured	Predicted (stability index)	
		D	C		D	C
4	900	500	740	445	160	440
12	1600(max.) 1150(av.)	1300	2000	260	300	1200

Influence of Topography Upon Plume Transport

It often appears, that plumes are guided to a degree by the topography of the underlying terrain; they seem to be ducted along valleys rather than across the parallel higher terrain, diverted to a noticeable extent from the direction of the prevailing wind upon encountering a valley, or deflected around higher terrain. We present here three examples of the apparent alteration of plume travel direction by terrain.

(1) On 30 August 1977 at 0615 MDT our research aircraft encountered the plume near Colstrip, followed it southeast about 16 km to the Rosebud Creek valley, where it appeared to divert toward the southwest. A vertical profile of the power plant plume near Lame Deer from 0645 to 0735 MDT was constructed, and then the plume was followed farther southwest along the Rosebud Creek valley as far as the town of Busby. The profiled plume edges and representation of approximate terrain contours are presented in Figure 2.14. Maximum deflections of constituent concentrations are shown in Figure 2.15. At 0600 MDT, the Miles City airport reported surface winds of 3.6 m sec^{-1} at 280° and 7 m sec^{-1} at 270° at 1830 m MSL. The record from our mobile laboratory at Hay Coulee shows the 0600 MDT wind at 270° and less than 2 m sec^{-1}. From approximately 2150 MDT, 29 August, to 0200 MDT, 30 August, the wind direction had been between 330° and 360° at Hay Coulee, with an apparent frontal passage about 2300. Maximum wind velocity when the front passed was about 8 m sec^{-1}, with 3-5 m sec^{-1} during the 1.5 hours following. Between 0200 and 0600 MDT the wind gradually veered to the west; velocity was less than 2 m sec^{-1}. The plume found at Lame Deer and Busby probably left the stacks between 2150 and 0200 MDT, being carried a few degrees east of due south. After a few hours travel at an average heading of 165°, the plume was deflected to the west by the Rosebud Creek valley and by the higher elevations surrounding Badger and Garfield Peaks (elev. 1348 and 1315 m respectively). Near Lame Deer, the shape of the plume also appears to have been affected by the valley of Lame Deer Creek (Figure 2.15), which flows into Rosebud Creek from the south.

TABLE 2.5. MAXIMUM AND BACKGROUND CONCENTRATIONS OF PLUME CONSTITUENTS, EXPRESSED AS MEANS AND ONE-SIGMA STANDARD DEVIATIONS

Location Downwind	Time (MDT)	NO (ppb)	NO^2 (ppb)	bscat (x10^{-4} m^{-1})	O^3 (ppb)	AN (x10^3 cm^{-3})	SO^2 (ppb)
1310 m MSL approx 5 km	0738-0755	296±112 (n=15)	< 40	1.31±0.32 (n=15)	approx. 0	16.8±4.7 (n=13)	87±55 (n=13)
Background 5 km	0738-0755	~5 ppb	< 5 ppb	0.21±0.05	65.8±1.6	~ 1.0	ND*
1220 m MSL approx. 16 km	0800-0818	49.5±20.0 (n=10)	13.9±5.2 (n=9)	0.50±0.08 (n=12)	15.4±5.9 (n=11)	5.2±4.1 (n=10)	35±6 (n=5)
Background 16 km	0800-0818	~5 ppb	< 5 ppb	0.20	67.8±1.2	~ 1.0	ND*

*Not detectable

(2) On 31 August 1977, for several hours following 0800 MDT, the wind direction at Hay Coulee was between 290° and 330°, with velocities averaging 5 m sec^{-1}, with gusts to 9 m sec^{-1}. During this time the wind recorded at stack height was 290°-330°, at 10 m sec^{-1}, gusts to 15 m sec^{-1}. A vertical profile of the plume 12 km downwind was constructed for the period 0915 to 1024 MDT; for the period 1024 to 1140 MDT a horizontal profile of the plume from 12 to 48 km downwind was made (Figures 2.7 and 2.6). Figure 2.8 summarizes the maximum change of the plume constituents at the various distances downwind, and gives the aircraft flight altitude and surface elevation. Along Beaver Creek, 34 to 48 km downwind, the elevations of the upland parallel to the valley are as much as 500 ft (152 m) higher than the valley floor; the plume was neatly centered along the valley. The Beaver Creek valley is oriented exactly so as to not require an alteration of the plume's average direction of travel, but in the situation where the wind direction varies over a 40° range, the probability of the plume centering on Beaver Creek valley without any ducting influence would have been very low. Thus, we consider this to be an instance of ducting.

(3) On 1 September 1977, winds aloft at 1300 MDT were 300°, 8 m sec^{-1}, gusting to 15 m sec^{-1}. Beginning at 1030 MDT, winds at Hay Coulee were 320° ± 30° at 7 m sec^{-1} average, with a very gradual change in direction to 360° by 1300 MDT. Wind direction stayed constant at 360° until 1540 MDT, when it moved to 340° for 45 min, then resumed the 360° for most of the time until about 1900 MDT. The research aircraft constructed a vertical plume profile near Hay Coulee from 1233 to 1240 MDT, then commenced a horizontal plume profile out to a distance of 45 km downwind, ending at 1500 MDT (Figures 2.5 and 2.4, respectively), several kilometers southwest of Ashland in the Tongue River valley. It appears that the plume was deflected to the east around the Badger and Garfield Peaks highland, then ducted slightly west of due south by the Tongue River valley, while the prevailing wind was neither westerly enough in the first instance, nor northeasterly enough in the second to have caused such a plume

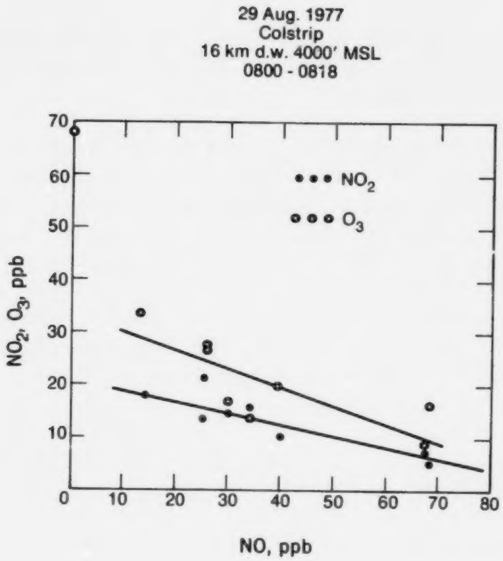

Figure 2.21. Least squares fit of NO_2 and O_3 vs. NO concentrations in the Colstrip plume in regions of different plume densities.

trajectory. In short, the evidence in this case indicates a deflection in the direction of plume travel by as much as 20° from the prevailing wind direction. This effect of terrain on plume dispersion introduces a complexity that cannot be explained by existing models.

Meteorology

Wind direction, wind velocity, relative humidity, and temperature recorded at the mobile laboratory at Hay Coulee were previously reported for the periods 27 August to 7 September 1976, 20 May to 4 June 1977, and 20 August to 3 September 1977 (Van Valin *et al.*, 1979). As a supplement to the ground-based observations the same parameters were measured at certain times within the given periods by aircraft, by radiosonde release, and by pilot balloon (pibal) release.

As Van Valin *et al.*, (1979) reported during the August to September periods the surface winds were typically light and variable at night, with daytime velocities of 2 to 6 m sec^{-1}, usually westerly. During the May-June periods, surface wind flow was less obviously diurnal, there was more cloudiness and more convective storm system activity. During all four periods nighttime and early morning inversions between 600 and 1200 m AGL with stability classes E and F were so common as to be almost the rule. Usually the change in wind direction and velocity with height became less distinct as surface heating took place during the day. Under the conditions of light and variable wind with the temperature inversion, the power plant plume rises to the inversion layer and then spreads out into a broad fan as it slowly moves away from the source area. This condition usually does not result in ground impact by the plume, save for the gravitational settling of particles. In fact, in the few hours preceding sunrise one often sees the formation of another inversion well below the stack height, which further insulates the surface from plume contact. The daytime counterpart during this diurnal regime involves the mid-morning development of convective activity, with concomitant looping of the plume and intermittent ground contact, followed by sufficient boundary layer heating to overcome temperature inversions and allow the plume to mix upward.

During frontal passage, with the cooler air, greater cloudiness, more persistent wind, and neutral temperature structure with "coning" of the plume occurs. Plume contact with the ground does not occur for some appreciable distance (on the order of several kilometers) but becomes rather constant with increasing distance, and is confined within an arc of roughly 20°. During stable temperature conditions with light-to-moderate wind, the plume may be carried horizontally with little vertical movement for long distances. This is the state that permits the best appraisal of plume dimensions and integrated loading, and represents meteorological conditions that exist during most of the airborne plume measurements dealt with in this report.

During winter, the fronts come more frequently, the average wind speed is greater, there is more cloudiness, and convective activity is reduced. There is also a greater frequency of long-lived low-level inversions; these are often below the level of the effective stack height and thus have the effect of shielding the surface from plume contact. Meteorological factors extant during the examples of plume measurement are included with the discussion of those measurements.

CONCLUSIONS

This research is a continuation of work reported previously (Abshire *et al.*, 1978; Van Valin *et al.*, 1979). It is the nature of research that continuing effort in a given direction, with new equipment and methods, not only goes forward using previous work and conclusions as a foundation, but also produces new information that modifies previous conclusions. This report includes new information that modifies the conclusions of the Fourth Interim Report in respect to the composition and size range of the plume aerosol, the formation of secondary aerosols, and the generation of IN. To the two types of particles reported previously for downwind distances of 10 km or more, i.e., a man-made aerosol consisting of spherical sulfate-containing particles in the 0.1-0.4 μm diameter range and a natural aerosol of irregularly shaped aluminosilicates of > 0.6 μm mean particle diameter, must now be added a third man-made aerosol consisting of nitrate-containing droplets mostly of 1-2 μm diameter. The formation of nitrate-containing droplets constitutes a significant sink for the NO formed in the power plant combustion chambers. In the Fourth Interim Report we concluded that IN concentrations, as measured by the acoustic counter method, were increased by an order of magnitude. Evaluation of IN concentrations by the filter method, however, shows no increase above background concentrations. We thus qualify the earlier conclusion by stating that potential IN are deactivated in relation to sublimation and condensation-followed-by-freezing modes of ice crystal formation, probably by surface absorption of sulfates, but that the contact mode of nucleation is affected to a much smaller extent.

Plume cross-section measurements at various distances downwind have shown the plume, under conditions of C or D stability, to be usually wider than predicted by Gaussian diffusion models. The necessary consequences of greater-than-predicted width is that a greater land area will be affected by deposition or by attenuation of solar radiation.

Plume trajectories have been found to be influenced by topography to such an extent that a plume's direction of travel is altered by as much as 20° from the direction of the prevailing wind. This introduces too much complexity for existing models of plume diffusion and transport.

REFERENCES

Abshire, N. L., V. E. Derr, G. T. McNice, R. Pueschel, and C. C. Van Valin. 1978. Integrated Aerosol Characterization Monitoring, Colstrip, Montana. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, E. M. Preston and R. A. Lewis, eds., EPA-600/3-78-021, U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 291-321.

Bigg, E. K., S. C. Mossop, R. T. Read, and N. S. C. Thorndike. 1963. The Measurement of Ice Nucleus Concentrations by Means of Millipore Filters. J. of Appl. Meteor. 2:266-269.

Bigg, E. K., A. Ono, and J. A. Williams. 1974. Chemical Tests for Individual Submicron Aerosol Particles. Atmos. Environ. 8:1-13.

Langer, G., J. Rosinski, and C. P. Edwards. 1967. A Continuous Ice Nucleus Counter. J. of Appl. Meteor. 6:114-125.

Mamane, Y., and R. G. dePena. 1978. A Quantitative Method for the Detection of Individual Submicrometer Size Sulfate Particles. Atmos. Environ. 12:69-82.

Mamane, Y., and R. F. Pueschel. 1979a. Oxidation of SO_2 on the Surface of Fly Ash Particles under Low Relative Humidity Conditions. Geophys. Res. Lett. 6, pp. 109-112.

Mamane, Y., and R. F. Pueschel. 1979b. A Method for the Detection of Individual Nitrate Particles. Atmos. Environ., accepted for publication.

Parungo, F., E. Ackerman, H. Proulx, and R. Pueschel. 1978a. Nucleation Properties of Fly Ash in a Coal-Fired Power-Plant Plume. Atmos. Environ. 12:929-935.

Parungo, F. P., P. A. Allee, and H. K. Weickmann. 1978b. Snowfall Induced by a Power Plant Plume. Geophys. Res. Lett. 5:515-517.

Parungo, F. P., R. F. Pueschel, and D. L. Wellman. 1980. Chemical Characteristics of Oil Refinery Plumes in Los Angeles. Atmos. Environ., 14:509-522.

Pueschel, R. F., F. P. Parungo, E. W. Barrett, D. L. Wellman, and H. Proulx. 1979a. Meteorological Effects of Oil Refinery Operations in Los Angeles. NOAA Tech. Memo. ERL APCL-22, 62 pp.

Pueschel, R. F., R. C. Schnell, H. K. Weickmann, and D. L. Wellman. 1979b. Aerosol and Ice Nuclei Measurements in the Plume of the Homer City, PA, Power Plant. Geophys. Res. Lett. 6:371-374.

Pueschel, R. F., and C. C. Van Valin. 1978. Cloud Nucleus Formation in a Power Plant Plume. Atmos. Environ. 12:307-312.

Schnell, R. C., C. C. Van Valin, and R. F. Pueschel. 1976. Atmospheric Ice Nuclei: No Detectable Effects from a Coal-Fired Power Plant Plume. Geophys. Res. Lett. 3:657-660.

Turner, D. B. 1970. Workbook of Atmospheric Dispersion Estimates. Public Health Service, 999-AP-26, 84 pp.

Van Valin, C. C., R. F. Pueschel, and F. P. Parungo. 1980. Sulfate and Nitrate in Plume Aerosols from a Power Plant Near Colstrip, MT. In: Environmental and Climatic Impact of Coal Utilization, J. J. Singh and A. Deepak, eds., Academic Press, New York, pp. 35-48.

Van Valin, C. C., R. F. Pueschel, D. L. Wellman, N. L. Abshire, G. M. Lerfald, and G. T. McNice. 1979. Aerosol Characterization in the Vicinity of Colstrip, MT. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, MT, E. M. Preston and T. L. Gullett, eds., EPA-600/3-79-044, Section 1, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 2-52.

SECTION 3

OBSERVATIONS OF TWO LICHEN SPECIES IN THE COLSTRIP POWER PLANT VICINITY

S. Eversman

ABSTRACT

Two lichen species, *Usnea hirta* (L.) Wigg. and *Parmelia chlorochroa* Tayl. appear to be exhibiting no adverse effects from SO_2 emissions from the Colstrip coal-fired power plant. Variations in percentages of algal plasmolysis, respiration rates, and relative chlorophyll extract absorbances do not have a pattern that can be readily attributable to power plant emissions.

INTRODUCTION

Sulfur dioxide is known to adversely affect lichens (LeBlanc and Rao, 1975; Eversman, 1978). Community analysis, generally documenting community simplification in polluted areas, has been the most common method of detecting or describing patterns of pollution (Gilbert, 1973; Nash, 1972). Since there were insufficient numbers of species for community analysis on the upper trunks and branches of ponderosa pines in the Colstrip area, the major approach of this study has been to observe two lichen species for physical and anatomical changes. Two species of lichens, *Usnea hirta* (L.) Wigg. and *Parmelia chlorochroa* Tayl., from the Colstrip power plant vicinity have been observed in the laboratory for indications of stress from power plant emissions, since coal-burning began in September, 1975.

MATERIALS AND METHODS

During 1978, *U. hirta* samples were collected from 15 ponderosa pine (*Pinus ponderosa* L.) sites 1-60 km from the Colstrip power plant. Monitoring sites within 10 km of Colstrip received *U. hirta* transplants from a generally east-facing site in Custer National Forest 60 km ESE of Colstrip (Site P10, Figure 3.1).

Ponderosa pine branches containing *U. hirta* were wired onto appropriate branches of ponderosa pines facing the power plant (Sites P1-8, P17-19). Sites P10-16 have abundant native *U. hirta*. Other transplants were made to sites P15 and P16 as transplant controls; *i.e.*, to observe possible effects of transplanting.

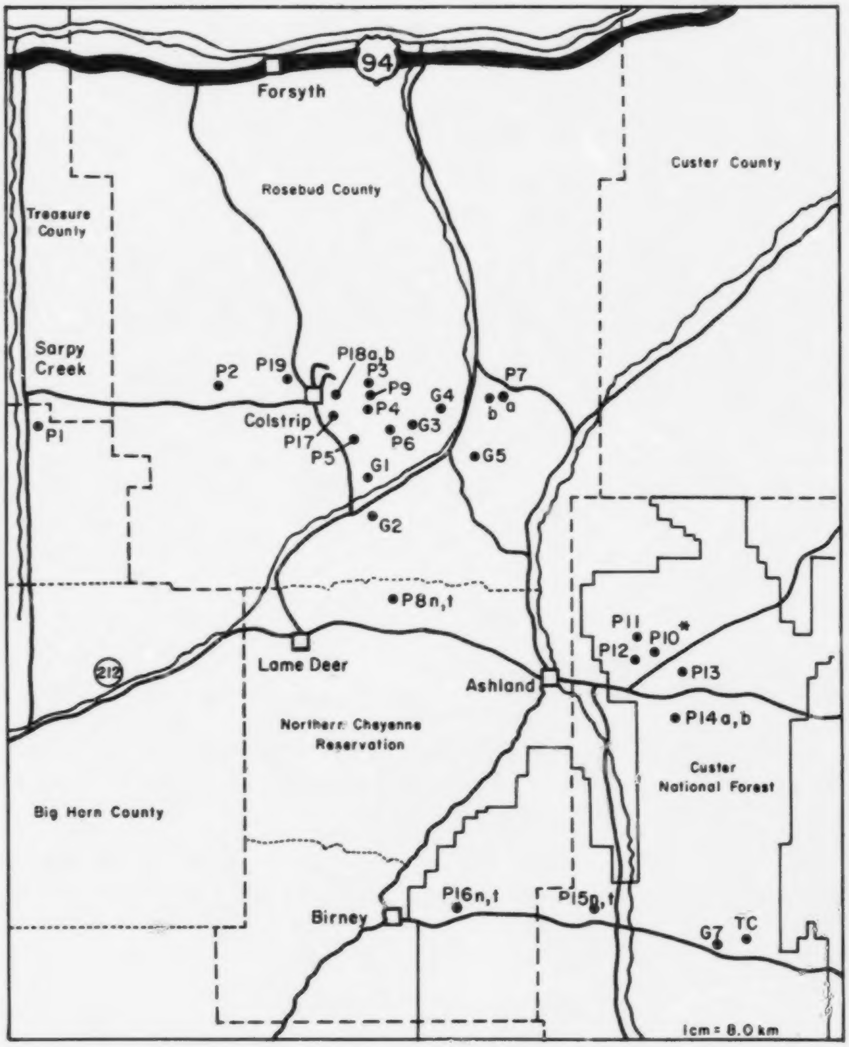
94
Forsyth
Rosebud County
Custer County
Treasure County
Sarpy Creek
P2
P19
P18a,b
P3
P9
P4
G4
P7
b
a
P1
Colstrip
P17
P5
P6
G3
G1
G5
G2
P8n,t
P11
P10*
P12
P13
212
Lame Deer
Ashland
P14a,b
Northern Cheyenne Reservation
Custer National Forest
Big Horn County
Birney
P16n,t
P15n,t
G7
TC
1cm = 8.0 km

Figure 3.1. Map showing lichen collection sites. P1-P16 are ponderosa pine sites with *Usnea hirta*; G1-G7 are grassland where *Parmelia chlorochroa* is collected. Sites P8, 15, and 16 have both native and transplanted *U. hirta* (n,t). Site P7 has two buttes (a, b); *U. hirta* is collected from the top and bottom of a hill at P14. P10* = *U. hirta* transplant source. TC = Taylor Creek fumigation sites (ZAPS).

Parmelia chlorochroa was collected from four grassland sites near EPA exclosures (G1-G4), three other sites near ponderosa pine sites (P3, P4, P18), and the control grassland site (G7).

Respiration rates were determined manometrically for all samples. Chlorophyll extracts were made with hot methanol and absorbance was recorded at 665 nm. Percentages of algal cell plasmolysis were determined according to previously described methods (Eversman, 1978).

RESULTS AND DISCUSSION

Neither *U. hirta* nor *P. chlorochroa* exhibited consistent visible signs of SO_2 injury in the Colstrip vicinity. Samples from sites closest to Colstrip were examined for algal plasmolysis rates compared with control levels. While some sites had a mean plasmolysis rate above control rates, differences were not significant (Figure 3.2). *Parmelia chlorochroa* from site G5 (Kluver

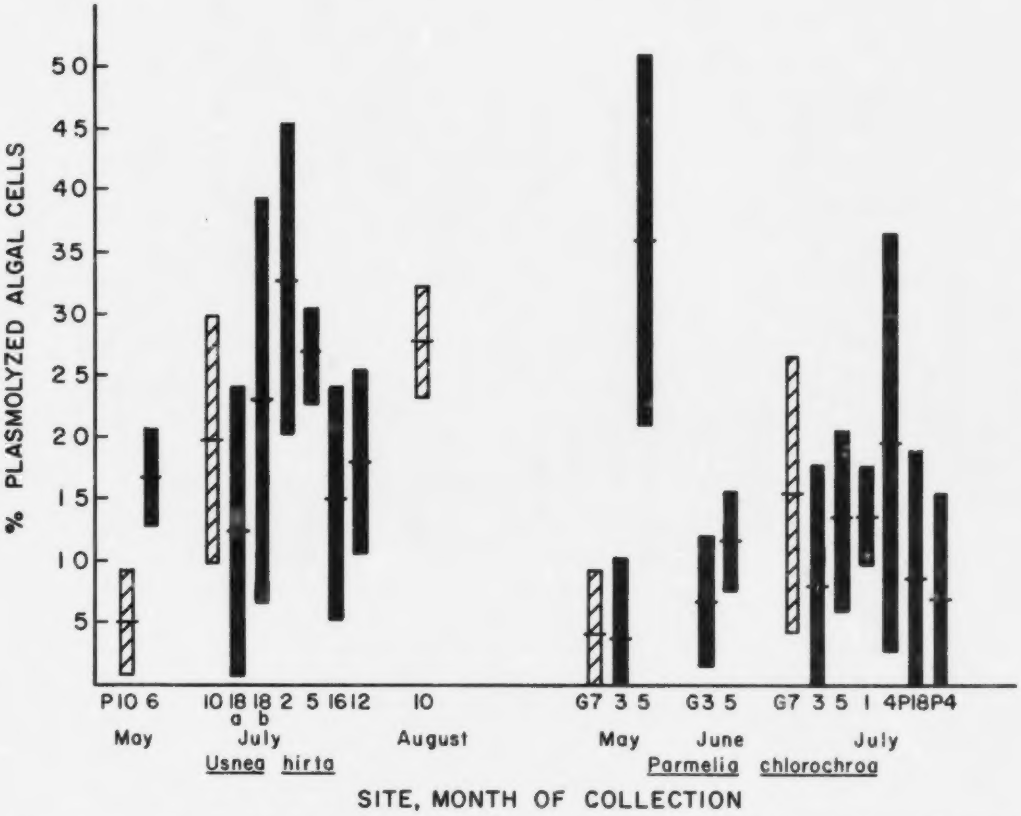

Figure 3.2. Percentages of algal plasmolysis of *U. hirta* and *P. chlorochroa* from monitoring sites. Percentages are means of three samples ± 0.95 confidence interval. Cross-hatched bars are control sites (P10, G7). Solid bars are field collection sites. Confidence intervals = t.$_{05}$(standard error)/$\sqrt{n}$ for each mean.

East) was exceptionally yellow and had an unusually high algal plasmolysis rate (35%) in May, 1978. However, by June the population appeared normal again; plasmolysis percentage dropped and remained as low as other samples during 1978. The general level of plasmolysis of samples from the Colstrip area was below the plasmolysis rates of samples exposed to known levels of SO_2 in ZAPS fumigation plot B (See Section 9).

Respiration rates in 1978 showed no pattern associated with distance from the power plant (Figure 3.3). Differences among sites appeared to be due to natural variabilities, not air pollution.

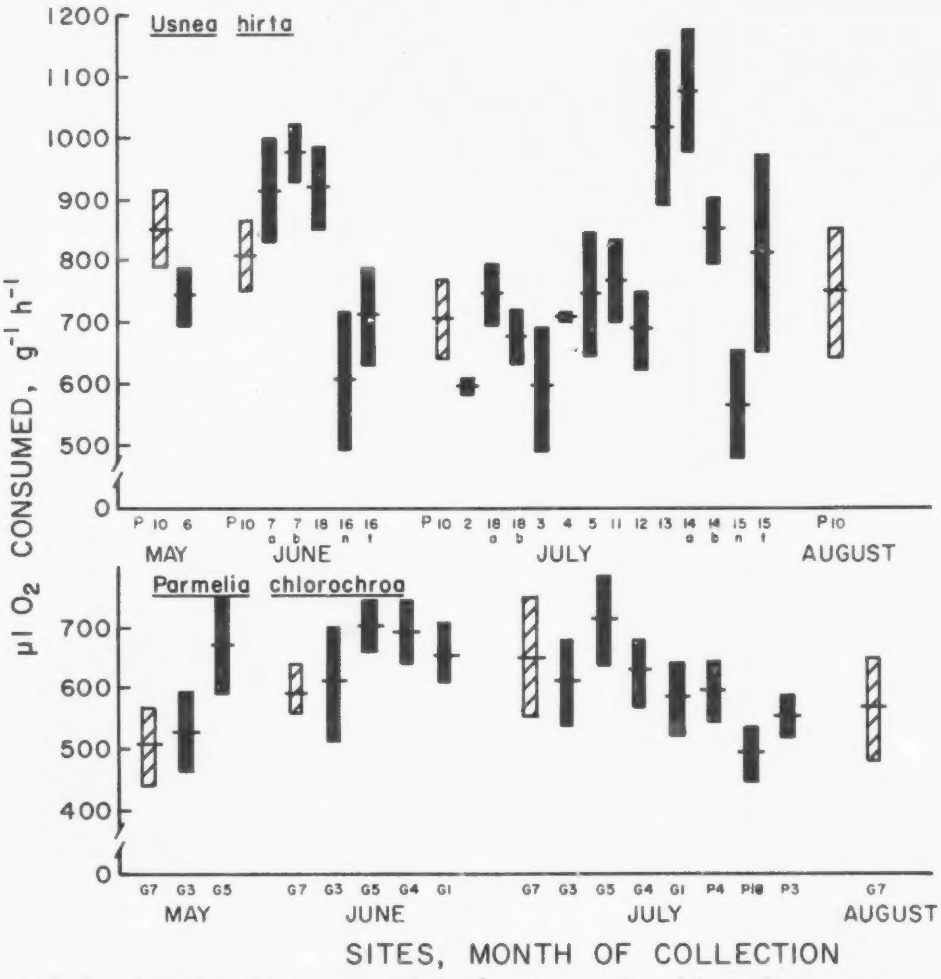

Figure 3.3. Respiration rates of *U. hirta* and *P. chlorochroa* from monitoring sites in the Colstrip area, 1978. Rates are given as means of five samples ± 0.95 confidence interval. Cross-hatched bars are control samples (sites P10, G7). Solid bars are monitoring sites. Confidence intervals = $t_{.05}$(standard error)/$\sqrt{n}$ for each mean.

Relative chlorophyll extract absorbance values for both species were generally not significantly less than control values (Figure 3.4), and they were higher than values obtained from samples from plot B on the ZAPS sites (Section 9). Differences, however, were not significant.

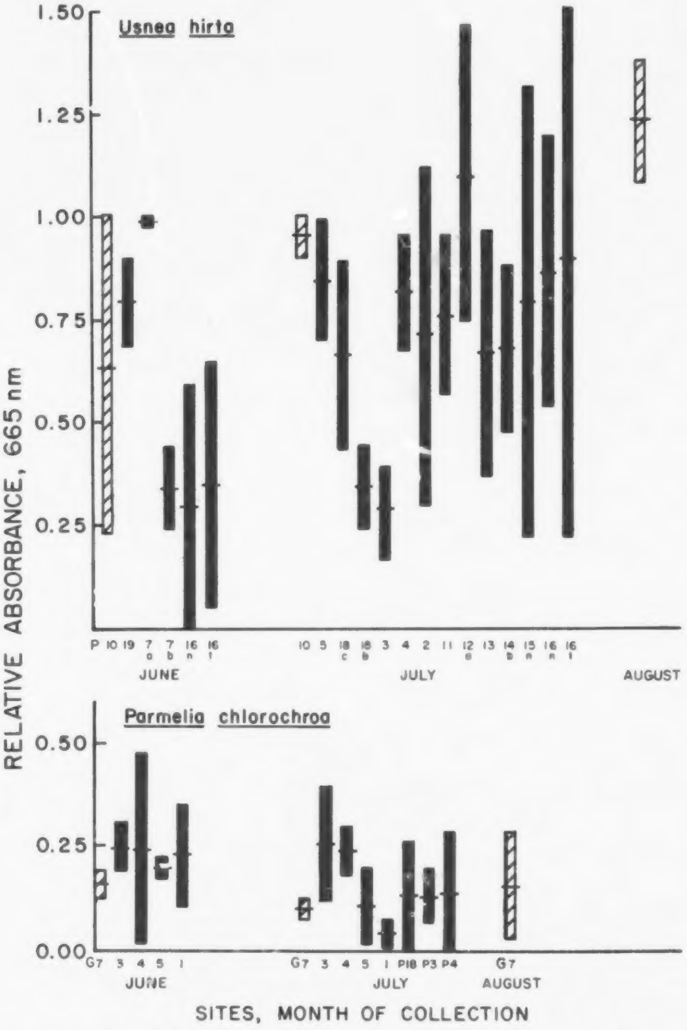

Figure 3.4. Relative chlorophyll extract absorbance at 665 nm for *U. hirta* and *P. chlorochroa*. Values are means of three samples ± 0.95 confidence intervals. Cross-hatched bars are controls (P10, G7). Solid bars are monitoring sites. Confidence intervals = $t_{.05}$(standard error/$\sqrt{n}$ for each mean.

Samples collected from the ZAPS site plots B, C, and D have consistently been bleached, with yellowing of the thallus occurring in 30-60 days after transplanting onto the fumigation plots (Eversman, 1978). No such yellowing has been observed in samples from the Colstrip area (with the one temporary exception in *P. chlorochroa* in May, 1978). All collections made during 1978 exhibited the "normal" green thallus color.

CONCLUSIONS

If impact from power plant emissions were occurring on the lichens, one would expect a gradient of respiration rate changes, decreased algal plasmolysis, increased relative chlorophyll extract absorbance, and decreased yellowing with increasing distance from the power plant. The two lichen species showed no such trends, and therefore did not appear to be affected by Colstrip power plant emissions in 1978.

REFERENCES

Eversman, S. 1978. Effects of Low-Level SO_2 on *Usnea hirta* and *Parmelia chlorochroa.* Bryologist 81: 368-377.

Gilbert, O. L. 1973. Lichens and Air Pollution. In: The Lichens, Ahmadjian, V. and M. Hale, eds. Academic Press, New York. pp. 443-472.

LeBlanc, F. and D. Rao. 1975. Effects of Air Pollutants on Lichens and Bryophytes. In: Responses of Plants to Air Pollution. Mudd, J. B. and T. T. Kozlowski, eds. Academic Press, New York. pp. 237-272.

Nash, T. H. III. 1972. Simplification of the Blue Mountain Lichen Communities Near a Zinc Factory. Bryologist 75: 314-324.

SECTION 4

PLANT COMMUNITY MONITORING IN THE VICINITY OF COLSTRIP, MONTANA

J.E. Taylor, W.C. Leininger and M.W. Hoard

ABSTRACT

Since 1974, plant community studies have been conducted near Colstrip, Montana on four exclosures established by the EPA and on three contiguous pristine knolls along Rosebud Creek. All study locations were within 20 kilometers of the coal-fired plants at Colstrip. Comparisons of species cover, diversity, and density from 1974 to 1978 indicated that a stabilization of grazing deferment effects occurs over time. This should allow better isolation of air pollution effects in the future. Canopy coverage varied substantially among sites due to the relative amounts of graminoids and lichens. Forbs did not vary markedly. Mosses and shrubs were not important. No trends in lichen cover can be ascribed to pollution because of the confounding effects of grazing deferment in earlier years. Western wheatgrass density varied among sites, but intra-site variation was slight, suggesting this species is a good monitoring organism. Species richness and evenness contributed about equally to species diversity. Standard errors for species diversity were mostly within 5% of mean values. Photographic plots were charted in detail and added to the long-term monitoring record.

INTRODUCTION

This research was begun July 15, 1974, to monitor bioenvironmental effects of coal-fired electric generating plants in southeastern Montana. The objective is to monitor the effects of stack emissions on native rangeland vegetation. This objective is being approached through canopy coverage and plant density transects and by aerial and ground-level photography and species diversity analysis.

The data reported in this section cover only our research activities in the vicinity of Colstrip, Montana. In addition to four exclosures established and maintained by the EPA, three relatively pristine knolls are being examined (Figure 4.1). Previous work and study site descriptions have been summarized by Taylor and Leininger (1977 and 1979).

MATERIALS AND METHODS

Plant community analysis and photographic monitoring are discussed in detail below.

Plant Community Analysis

Canopy Coverage

Canopy coverage estimates were made on all study sites near the middle of August, 1978. Sample plots within study areas were located by placing a cord with meter-spaced knots in a random meandering pattern through the sample area. Then 2 x 5 dm plot frames were placed at each knot and canopy coverage estimated using the procedures of Daubenmire (1959). Preliminary studies showed that 2 lines of 25 frames each provided the optimum sampling efficiency.

Western Wheatgrass Density

In the same samples which were used for canopy coverage, numbers of western wheatgrass plant units were determined. Plant units were defined as single or clustered vegetative and/or reproductive stems which appeared to originate at a common basal node. Thus, a reproductive culm closely surrounded by one or several vegetative culms was considered a plant unit, and an isolated vegetative culm was a plant unit.

Species Diversity

The diversity index used was the Shannon-Weaver function (Shannon and Weaver, 1949):

$$H' = -\sum_{i=1}^{S} \frac{N_i}{N} \log_2 \frac{N_i}{N}$$

Where H' = Index of diversity
S = number of species
N_i = canopy of ith species
N = total canopy of all species

Percent canopy estimates were used to calculate diversity.

In addition to diversity, these data were used to calculate evenness (equitability) and species richness.

Evenness was calculated with the equation of Pielou (1969).

$$J' = \frac{H'}{\log_2 S}$$

Where J' = Evenness
H' = Shannon-Weaver function
S = total number of species

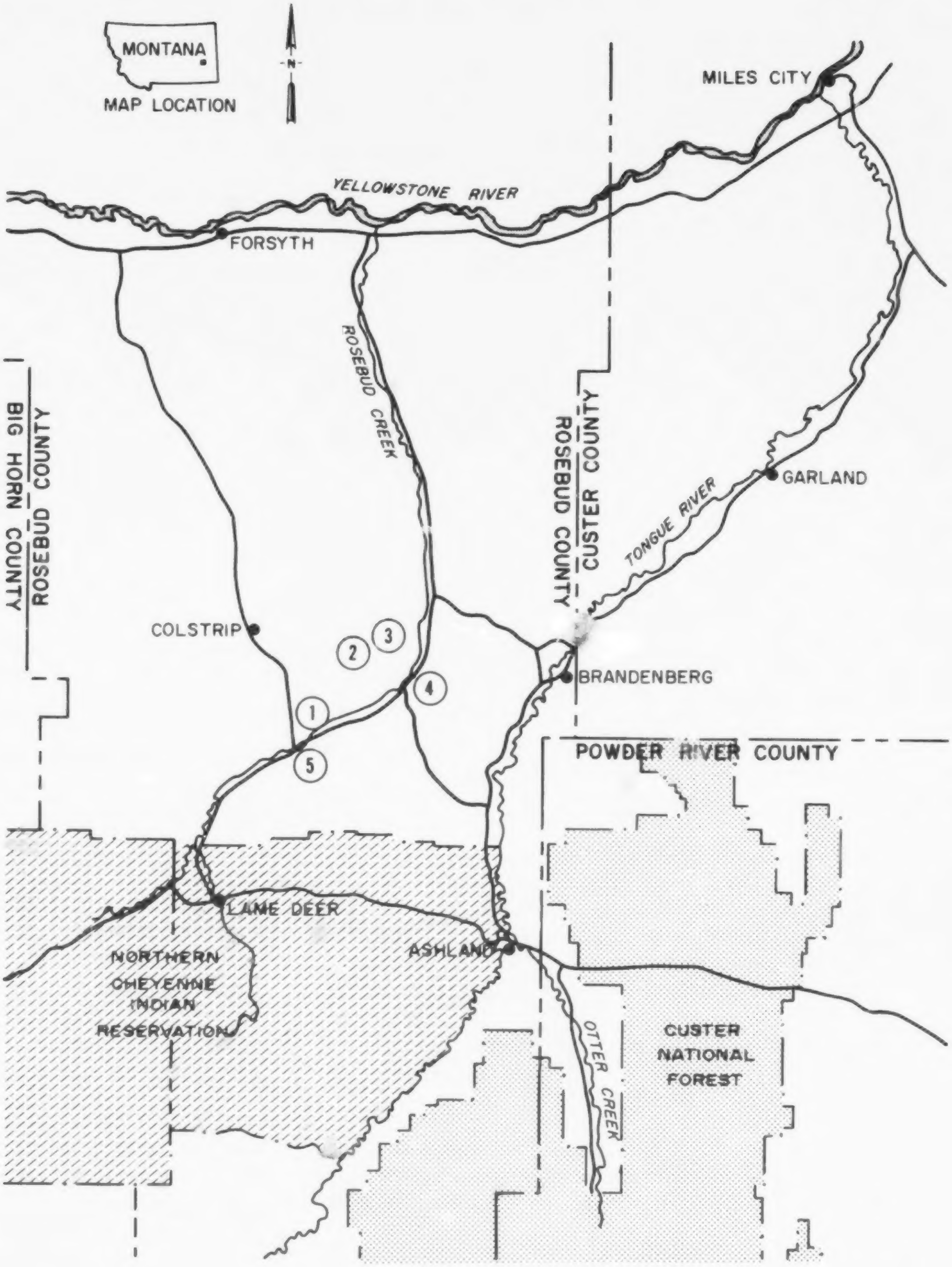
MONTANA
MAP LOCATION
N
MILES CITY
YELLOWSTONE RIVER
FORSYTH
ROSEBUD CREEK
BIG HORN COUNTY
ROSEBUD COUNTY
ROSEBUD COUNTY
CUSTER COUNTY
GARLAND
TONGUE RIVER
COLSTRIP
2
3
4
1
5
BRANDENBERG
POWDER RIVER COUNTY
LAME DEER
NORTHERN CHEYENNE INDIAN RESERVATION
ASHLAND
OTTER CREEK
CUSTER NATIONAL FOREST

Figure 4.1. Study sites in the vicinity of Colstrip. (1 = Hay Coulee, 2 = Kluver West, 3 = Kluver North, 4 = Kluver East, 5 = McRae Knolls)

Species richness is the numerical sum of species which were used to calculate H'.

In order to reduce variances and still be able to calculate standard errors, data were grouped into five consecutive frame sums before H' or J' were calculated. S was the total number of species encountered in each five-frame group.

Photographic Studies

Ground-level photography provides a detailed record of plant species, phenology, and pathologic signs. This assists in the interpretation of aerial imagery. Also, vertical ground photo plots may be measured and analyzed for cover, number, frequency, pattern, and plant volume. Plant volume, which can be used to estimate biomass, may be obtained by combining canopy coverage and height, the latter measured with a parallax wedge. Plant density and pattern also can be studied from these pictures.

At the Hay Coulee and Kluver sites, two photo plots were established in each exclosure, while one photo plot was placed on each of the three McRae knolls.

The photo plots are 1 meter square in area, and are marked for relocation. Each is photographed in color and black-and-white film emulsions. Stereoscopic photography is used for ease of plant identification. Most of the plots have also been photographed with infrared color film. More details are given by Taylor *et al.*, 1976.

Aspect photographs are made from vantage points within and overlooking study areas. These are taken with color and infrared color film, the latter to compare with aerial coverage.

For each photo plot an index of species identification has been prepared. During summer, 1978, precise maps were drawn of each photo plot. Each square-meter plot was divided into a 10 x 10 grid, each unit a square decimeter. Within each grid cell, plant species with substantial area were delineated; smaller plants were indicated symbolically, and numbers of western wheatgrass plant units were recorded.

The combination of plot photographs and plot indices makes a permanent record of species presence and distribution. Sequential records allow the evaluation of temporal changes.

During 1978, no new aerial photography was acquired in the Colstrip area. Color photography from the helicopter flight in 1977 was used to develop detailed vegetation maps of the Hay Coulee and Kluver sites.

RESULTS AND DISCUSSION

Plant Community Analysis

Canopy Coverage

The generalized plant composition among study sites is shown in Figure 4.2. Species vary, reflecting differences in sites, microclimates, and previous use. Therefore, plant group data are presented, which more clearly portray inter-site variation.

Total canopy coverage varies among sites, primarily reflecting differential site potentials. More significant differences may be observed by considering the relative coverages of plant groups within sites.

The patterns of variation in total cover were similar to those of 1977. This suggests that inherent site differences may be replacing deferment effects as the predominant influences on plant species composition. If this is the case, 1978 and later observations should begin to show any pollution effects more clearly than earlier data, since the relative confounding influence of deferment will be diminished.

As should be expected in these vegetation types, graminoids dominate on all sites. The differences among sites which were observed in 1977 had essentially disappeared in 1978. This reflects the more favorable growing season in 1978 and the lesser influence of grasshoppers. The canopy cover of graminoids ranges from 39 to 59 percent across the sites.

Forb cover is remarkedly uniform among sites. The lowest and highest forb covers recorded were 9 and 14 percent, respectively. There were no particularly conspicuous forb species associated with sites. Shrub and moss cover likewise was considered unimportant. Mosses never were conspicuous, and shrubs were clearly a function of pre-treatment distribution. 1978 canopy coverage values are presented in Table 4.1.

With the initiation of the Colstrip studies in 1974, pre-treatment baseline vegetation data collecting was begun within newly established grazing exclosures. Comparable grazed plots were not established with available resources because there was no way to quantify grazing in ways in which grazing would be the only variable inside and outside exclosures.

The lack of grazed controls has caused some problems in the interpretation of data from the exclosures. By 1975 it was clear that exclosure effects (ie., grazing deferment effects) were producing substantial increases in plant growth. This was particularly marked on the Kluver East site, which had previously been used as a feeding area for horses and bulls and as a season-long pasture. Under protection from livestock grazing, the dominant vegetational aspect changed to crested wheatgrass, apparently from residual seed which had come from grass hay and which had been trampled into the soil.

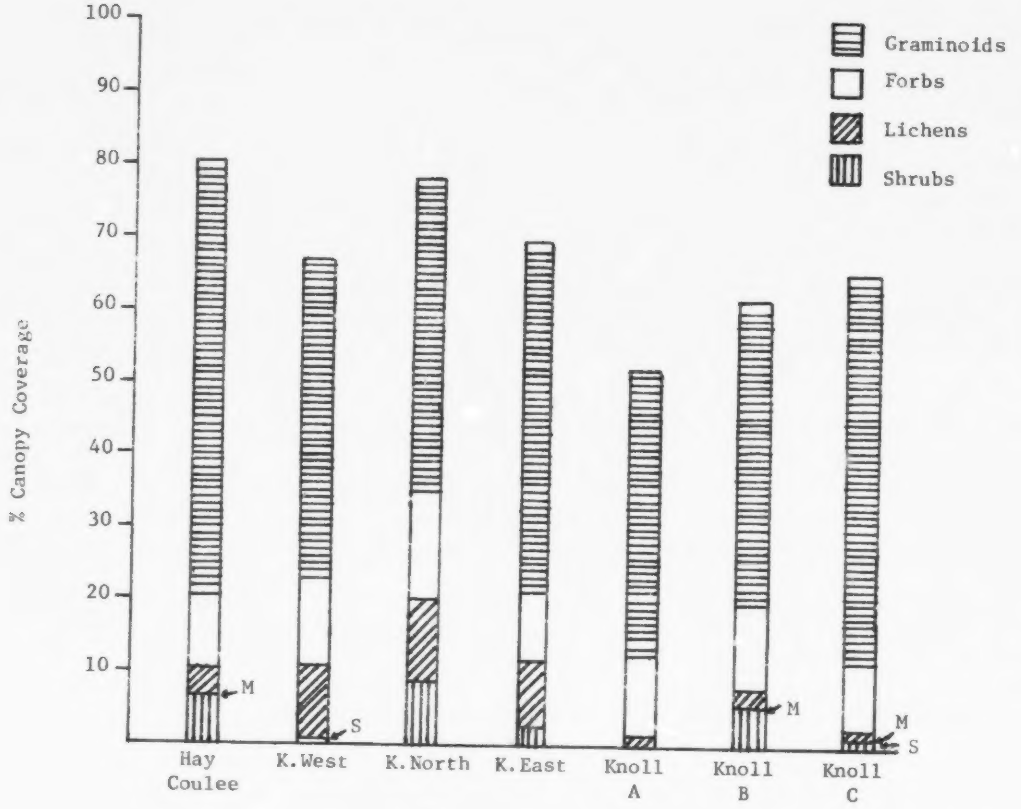

Figure 4.2. Canopy coverage on the Colstrip sites, August, 1978.

This rejuvenation of plant vigor greatly over-compensated for any air pollution effects. Thus, yield, or even species composition changes have been impossible to positively ascribe to either pollution or deferment, since these factors are confounded. The vegetation which exists in the exclosures is the product of deferment and of any air pollution influences which may exist, as well as soil, climate, weather variation, previous grazing, and all the other many interrelated variables which function in rangeland vegetation communities.

To further cloud this issue, the research which has been conducted on the ZAPS sites has only recently shown subtle signs of incipient effects of SO_2 pollution, and there the levels of pollutants are higher and more continuous than those likely to be experienced in power plant situations.

In the long term, species composition changes should follow pollution stresses, and each year's data collection will help to characterize the natural amplitude of plant growth and vigor which respond to both climatic variation and pollution. Meanwhile, more restrictive indicators must be

TABLE 4.1. CANOPY COVERAGE (PERCENTAGE) FOR COLSTRIP STUDY SITES, 1978

SPECIES	Hay Coulee 20 Aug	Kluver West 17 Aug	Kluver North 16 Aug	Kluver East 18 Aug	McRae Knoll A 19 Aug	McRae Knoll B 19 Aug	McRae Knoll C 18 Aug
GRAMINOIDS							
Agropyron cristatum				29.50			
A. smithii	11.50	4.20	6.40	6.25	2.45	2.40	4.35
A. spicatum						7.30	
Aristida longiseta		.35		.05		.45	
Bouteloua gracilis	27.95	3.45	6.55	1.75	1.15	6.00	2.40
Bromus japonicus	2.50	2.50	2.20	2.75	2.75	1.70	4.35
B. tectorum	.65	1.60	.30		1.05		1.75
Calamovilfa longifolia						4.40	2.75
Carex filifolia		1.55			13.85	4.55	23.45
Koeleria cristata	8.55	.40	.05	.30	1.00	10.10	.75
Poa pratensis	.60			.05	.35		
P. sandbergii	4.55	3.50	7.20	2.40	.40		.30
Schedonnardus paniculatus				.10			
Sporobolus cryptandrus		.30		.05			
Stipa comata	1.30	26.05	17.70	4.85	15.10	5.15	13.25
S. viridula	.75		2.05			.05	
Vulpia octoflora	.55	.30	.25		.45		.20
FORBS							
Achillea millefolium				.30	.65	.10	
Alyssum desertorum	.70		.10				
Ambrosia psilostachya							1.90
Androsace occidentalis	.30	.85	.40	1.30	.80		.25
Antennaria rosea		.10					
Arabis holboellii					.10		
Aster sp.			.10		.10		
Astragalus sp.					.05	.40	.05
Camelina microcarpa				.05			

TABLE 4.1 (continued)

SPECIES	Hay Coulee 20 Aug	Kluver West 17 Aug	Kluver North 16 Aug	Kluver East 18 Aug	McRae Knoll A 19 Aug	McRae Knoll B 19 Aug	McRae Knoll C 18 Aug
FORBS (continued)							
Comandra umbellata						.50	
Conyza canadensis		.05	.05	.05	.05		
Draba sp.	.05						.05
D. nemerosa				.35	.15	.10	
Erysimum asperum	.05	.15	.30		.45		.30
Erigeron divergens	.05						
Evolvulus pilosus		.35	.05	.05			
Gaura coccinea							.75
Gilia congesta						.30	
Grindelia squarrosa			.20			.05	
Heterotheca villosa	.80	1.50	1.00	.15			
Lactuca serriola			.05		5.10	.05	.05
Liatris punctata	.10						
Lygodesmia juncea		3.00			1.45		3.00
Mammillaria missouriensis		.05			.05		
Medicago sativa						3.70	1.20
Opuntia fragilis		.35	.10	.35			.30
O. polyacantha			.30		1.15	.05	.05
Phlox hoodii	.85	.10		.20		.60	
Plantago patagonica	.10	1.05	.80	.15	.10	.20	
P. spinulosa	1.55	.05		.10			
Polygala alba					.40	2.50	.05
Polygonum viviparum			.05				
Psoralea argophylla		.15		.05		.50	
Sphaeralcea coccinea	.40	.90	.45			.70	.25
Taraxacum officinale	.90		.30	.25	.70	.10	
Tragopogon dubius	5.05	3.00	10.20	5.95	.60	1.90	.35
Miscellaneous forbs				.05	.05		.70

TABLE 4.1 (continued)

SPECIES	Hay Coulee 20 Aug	Kluver West 17 Aug	Kluver North 16 Aug	Kluver East 18 Aug	McRae Knoll A 19 Aug	McRae Knoll B 19 Aug	McRae Knoll C 18 Aug
HALF SHRUBS AND SHRUBS							
Artemisia dracunculus			.30				.30
A. frigida	3.00	.60	8.40	2.90		3.90	1.10
A. tridentata	3.85						
Ceratoides lanata						1.50	
Chrysothamnus nauseosus						.05	
Xanthocephalum sarothrae						.30	
OTHERS							
Bare ground	12.40	16.50	16.50	13.90	6.85	25.10	13.00
Litter	75.85	60.85	62.95	69.55	79.00	57.50	69.55
Moss	.05					.45	.45
Lichens	3.50	10.40	11.95	9.15	1.65	2.05	.95
Rock	.15	.75	.20	.15	.25	2.30	1.70
TOTAL VEGETATION	80.20	66.85	77.70	69.55	52.15	61.65	65.60
TOTAL GRAMINOIDS	58.90	44.20	42.70	48.05	38.55	42.10	53.55
TOTAL FORBS	10.90	11.65	14.35	9.45	11.95	11.30	9.25
TOTAL SHRUBS	6.85	.60	8.70	2.90		5.75	1.40

used to evaluate pollution effects. The most likely approaches to use in this connection are to seek sensitive indicator groups or species and look for physiological effects or direct pathological signs which can be definitely ascribed to pollution. Of these, the most useful in this work has been the former, using lichens as indicator species.

Many species of lichens have long been considered to be sensitive indicators of pollution stresses, both in the scientific literature and in the CFPP study (LeBlanc and Rao, 1975; Eversman, 1978 and 1979; Taylor and Leininger, 1979).

Figures 4.3 and 4.4 show the lichen cover for 1975 prior to the operation of the power plants. The data show the lichen response to seasonal changes in the absence of air pollution. The trend is for lichen cover to decrease as the season progresses. This corresponds to the dying or maturing of vegetation, loss of soil moisture, and more difficult growing conditions with the advancing season. Some sites have more lichen growth than others. Sites that have recently been grazed (Figure 4.3) tend to have short vegetation and abundant bare ground where lichen growth thrives, especially in early season. On sites where recent grazing had not taken place (Figure 4.4), lichen levels were lower in early season and the trend toward reduction in plant population as the season progressed was less pronounced. This is probably caused by the increased vegetative volume that restricts available open space for lichen growth and shields lichens from seasonal shifts in microclimate.

Figure 4.5 and 4.6 show the yearly lichen cover variations from 1974 to 1978. Since data were gathered only in late August or early September in 1976-1978, the late season samples for 1974 and 1975 were used in these comparisons.

The sites with recent grazing history (Figure 4.5) show variability in cover between years. The variation between data collected in 1974 and 1975 (prior to power plant operation) probably reflects annual differences in growing conditions. The significant increases in lichen cover during 1976 may be due to favorable growing conditions. In 1977 and 1978 canopy coverage appeared to be stabilizing except for the high-level cover observed at the Kluver East site in 1977. This may have been caused by the atypical vegetational composition on that site composed of crested wheatgrass rather than a native mixture as on the other locations.

The sites with no recent grazing history (Figure 4.6) show less variability in cover between years. Lichen cover may gradually stabilize following grazing deferment.

Western Wheatgrass Density

Western wheatgrass density was measured in 1978 for several reasons. It is one of the principal forage species in the Northern Great Plains for both domestic and wild animals. It is found on numerous kinds of sites. Its

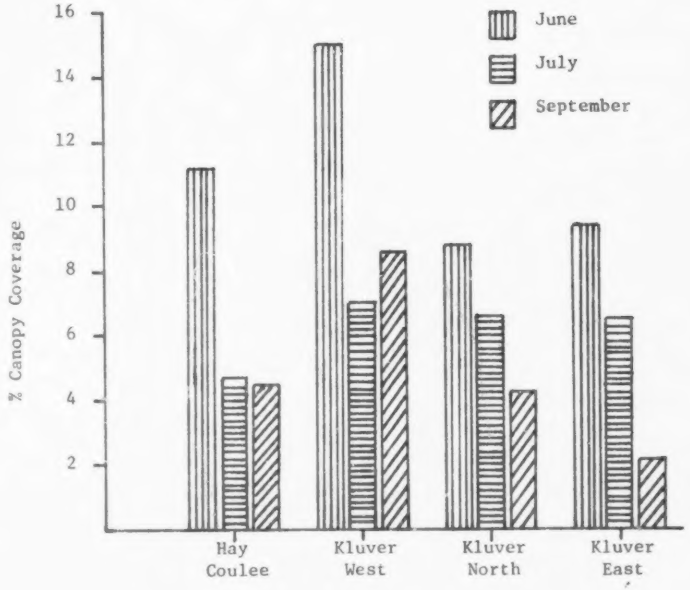

Figure 4.3. Seasonal changes in lichen coverage on the EPA established sites near Colstrip, 1975.

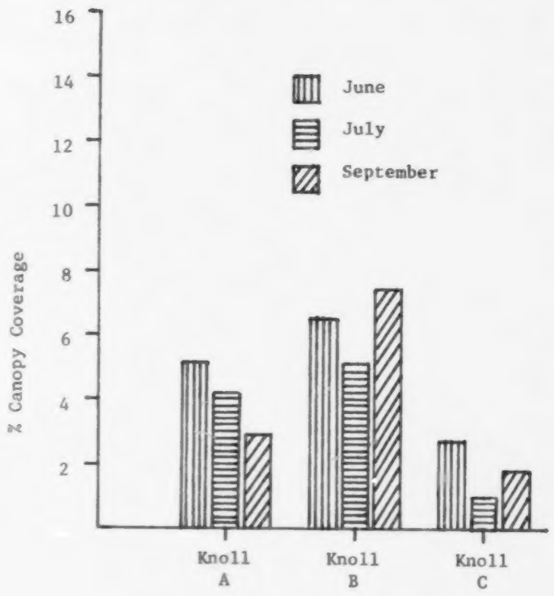

Figure 4.4. Seasonal patterns in lichen coverage for the McRae Knolls, 1975.

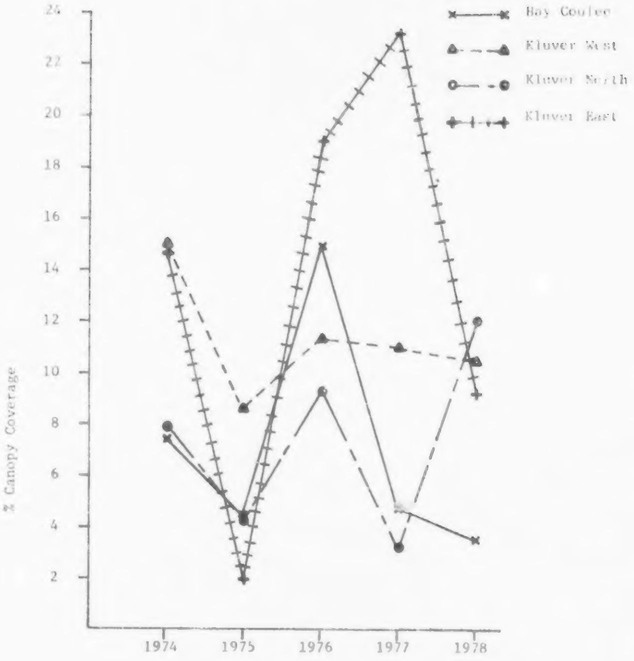

Figure 4.5. Lichen coverage on the EPA sites near Colstrip, 1974-1978.

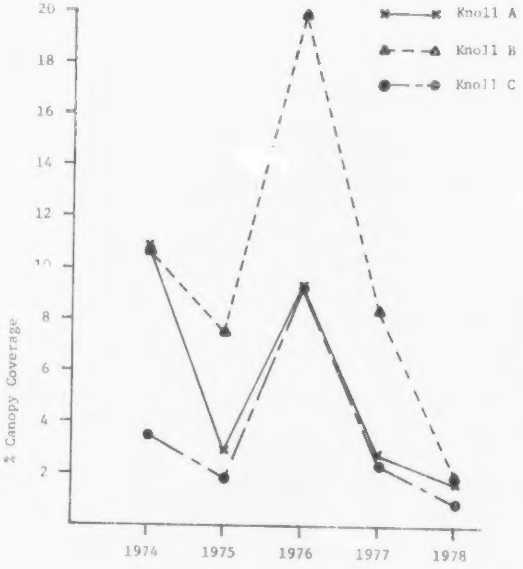

Figure 4.6. Lichen coverage near Colstrip taken late in August or early September, 1974-1978.

rhizomatous growth form allows direct comparisons among its density, cover, and weight. Finally, it has been studied in considerable detail by other project elements (Dodd *et al.*, 1979; Rice *et al.*, 1979; Heitschmidt, 1977).

In 1978, western wheatgrass density varied widely among sites (Figure 4.7) ranging from 2 to 19 plant units per 1/10 square meter. Although inter-site variation is high, intra-site variability is low, so temporal changes should be easy to detect when the anayses are completed.

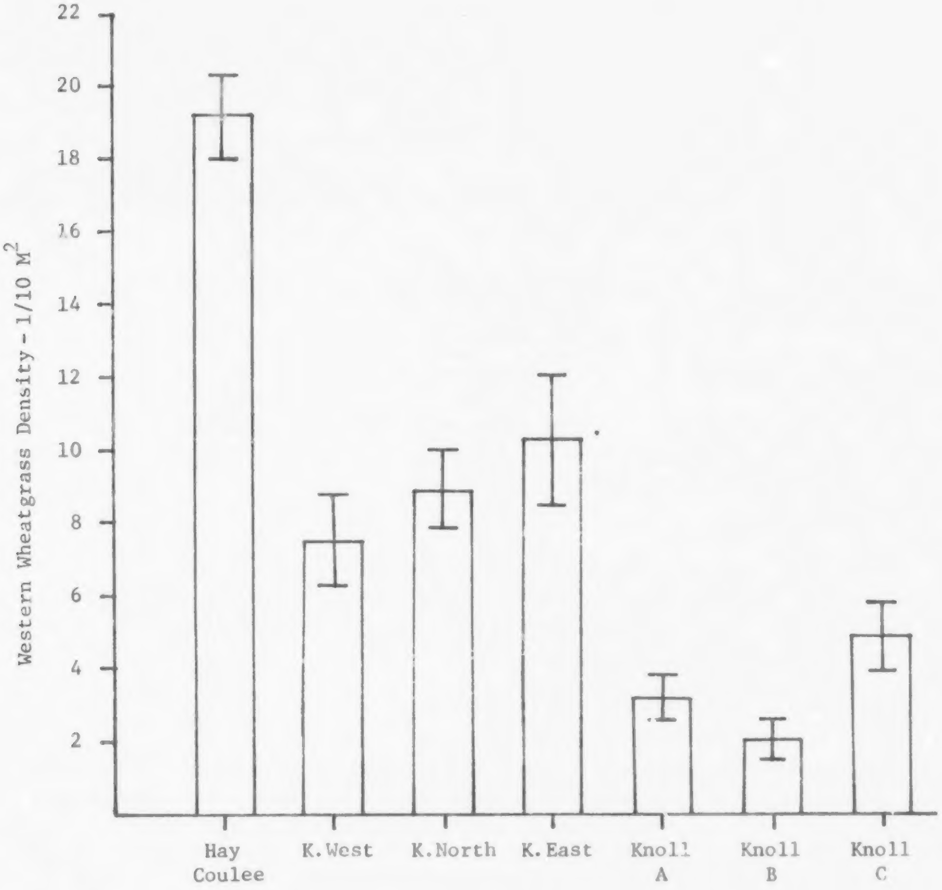

Figure 4.7. Density of Western wheatgrass on the Colstrip sites, 1978. (Mean value ± 1 standard error).

Species Diversity

Plant species diversity, evenness, and species richness for 1978 are shown in Figure 4.8 and 4.9.

As in past years, the diversity is highest at Hay Coulee and McRae Knoll B, and lowest at Kluver East. With the exception of Kluver East and Knoll B the values are quite comparable. The relationship between range condition and diversity, which has been previously discussed by Taylor *et al.*, (1975), is evident with Knoll B being the most diverse and also having the highest range condition and Kluver East being the least diverse, with the lowest condition. Standard errors are within 5% of the mean for all locations but Kluver East. The high variability in this site can be largely attributed to the great difference in diversity between the north and south replicates, caused by the uneven distribution of crested wheatgrass.

Species richness and evenness parallel diversity and appear to contribute about evenly to it (Figure 4.9). The high evenness value for the Kluver West site pulls up the diversity on the site even though it has a very low richness.

Diversity, richness, and evenness continue to be sensitive measures of community differences.

Photographic Studies

All permanent photo plots were photographed and mapped in August. These photos and plot maps are on file and constitute an important record of species composition, coverage, etc. Aspect photos of the sites were taken at the same time.

Site maps for the Kluver plots were also constructed in August and will be presented in the final report. McRae Knoll study sites were mapped the previous year. No aerial photography in the vicinity of Colstrip was obtained by our project in 1978.

CONCLUSIONS

Comparison of species cover, diversity, and density from 1974 to 1978 indicates that a stabilization of deferment effects occurred over time. Study of air pollution effects at the plant community level is impractical until stabilization is complete, but individual species and population responses can be meaningful. Species diversity, richness, evenness, and species composition all show sensitivity to site differences. Total canopy coverage is largely determined by graminoid cover and varies substantially among sites. Forbs do not vary markedly. Mosses were uncommon and shrub cover was clearly a function of pre-treatment distribution. Lichens constitute a small percentage of the biomass. We have not shown lichen cover to be a function of air pollution, perhaps because of confounding influences of grazing deferment and weather. Western wheatgrass density varies little

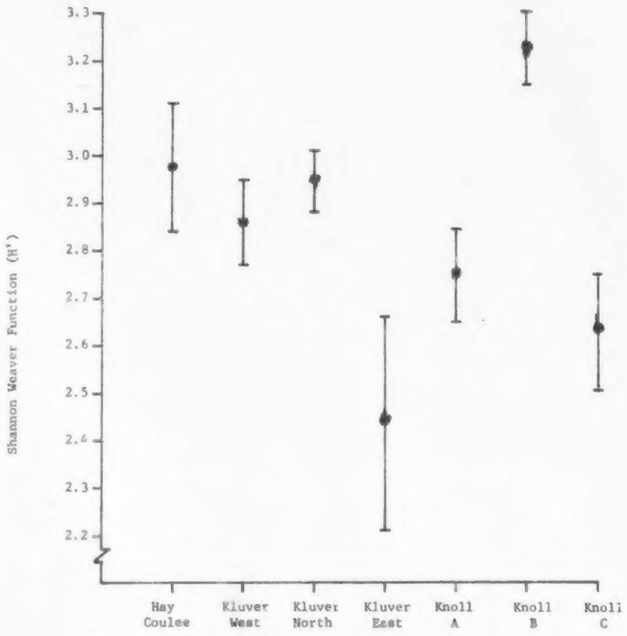

Figure 4.8. Diversity (H') for seven monitoring sites in the vicinity of Colstrip, 1978 (mean value ± 1 standard error).

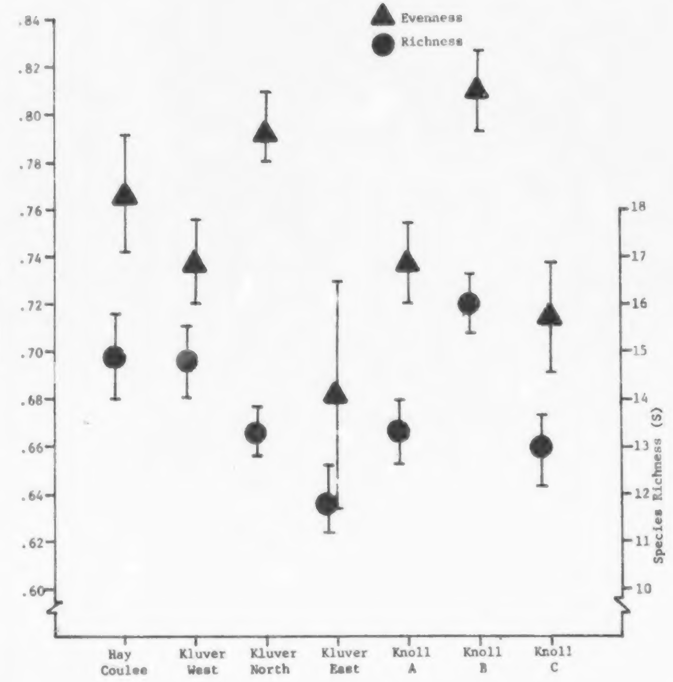

Figure 4.9. Evenness and species richness on the Colstrip monitoring plots, 1978 (Mean value ± 1 standard error).

within sites, but showed significant inter-site variation. Since it is an ubiquitous and important forage species in the Northern Great Plains, it may be a key species for monitoring pollution effects.

REFERENCES

Daubenmire, R. F. 1959. A Canopy-Coverage Method of Vegetational Analysis. Northw. Sci., 33(1):43-64.

Dodd, J. L., W. K. Lauenroth, G. L. Thor, and M. B. Coughenour. 1979. Effects of Chronic Low Level SO_2 Exposure on Producers and Litter Dynamics. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA 600/3-79-044. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 384-493.

Eversman, S. 1978. Effects of Low-Level SO_2 Stress on Two Lichen Species. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana. E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U. S. Environmental Protection Agency, Corvallis, Oregon, pp. 385-398.

Eversman, S. 1979. Effects of Low-Level SO_2 on Two Native Lichen Species. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds., EPA-600/3-79-044, U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 642-672.

Heitschmidt, R. K., 1977. Chronic Effects of SO_2 on Western Wheatgrass in a Montana Grassland. Ph.D. Thesis, Colorado State University, Fort Collins, Colorado. 100 pp.

LeBlanc, F. and D. N. Rao. 1975. Effects of Air Pollutants on Lichens and Bryophytes. In: Mudd, J. B. and T. T. Kozlowski (Eds). Response of Plants to Air Pollution. Academic Press, New York. pp. 237-272.

Pielou, E. C. 1969. An Introduction to Mathematical Ecology. J. Wiley & Sons, N.Y. 286 pp.

Rice, P. M., L. H. Pye, R. Boldi, J. O'Loughlin, P. C. Tourangeau, and C. C. Gordon. 1979. The Effects of "Low Level SO_2" Exposures on Sulphur Accumulation and Various Plant Life Responses of Some Major Grassland Species on the ZAPS Sites. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA 600/3-79-044. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 494-591.

Shannon C. and W. Weaver. 1949. Mathematical Theory of Communication. Univ. Illinois Press, Urbana. 117 pp.

Taylor, J. E. and W. C. Leininger. 1977. Baseline Vegetational Studies near Colstrip, Montana. Montana State University Mimeo. 67 pp.

Taylor, J. E. and W. C. Leininger. 1979. Plant Community Changes Due to Low Level SO_2 Exposures. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA 600/3-79-044. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 610-641.

Taylor, J. E., W. C. Leininger, and R. J. Fuchs. 1975. Baseline Vegetational Studies near Colstrip. In: Proc. Ft. Union Coal Field Symp., Mont. Acad. Sci., Billings, Montana. pp. 537-551.

Taylor, J. E., W. C. Leininger, and R. J. Fuchs. 1976. Monitoring Plant Community Changes due to Emission from Fossil Fuel Power Plants in Eastern Montana. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana, June, 1975. R. A. Lewis, N. R. Glass, and A. S. Lefohn, eds. EPA/600-3-76-013. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 14-40.

SECTION 5

ACCUMULATION AND TRANSFER OF FLUORIDE AND OTHER TRACE ELEMENTS IN HONEY BEES NEAR THE COLSTRIP POWER PLANTS

J. J. Bromenshenk

ABSTRACT

Coal-fired power plants at Colstrip, Montana went on line in 1975 and 1976. Fluoride levels in and on the body tissues of honey bees collected in 1978 were significantly higher at several locations than in 1974 and 1975. Mean fluoride increased at apiaries within 20 km downwind of Colstrip, especially in 1976 when mean levels at some beeyards were more than double baseline levels. Mean fluoride levels generally were lower in 1977 than 1976, although not as low as baseline levels. Levels in 1978 were higher than those of 1977 but not as high as 1976. There were significantly higher distributions of fluoride concentrations for each post-operational year compared to the pre-operational fluoride distributions in bee populations. Fluoride levels in bees from pristine areas and from fluoride exposed areas (via water and air) were highest in adult foragers, lower in hive bees, and lowest in the pupae. Preliminary data indicates that fluoride levels in or on soft body tissues were not significantly different from those of the hard exoskeletons of bees. Arsenic content of the bodies of worker honey bees in most cases was less than 0.8 ppm on a dry weight basis for all years (1974 through 1977). At an apiary 13 km northeast of Colstrip, 1.3 ppm arsenic was observed in 1977. This is twice the mean for 1977 and similar to levels observed in bees exposed to arsenic from a smelter near Butte, Montana. Accumulation as well as marked seasonal and site differences of arsenic, fluoride and lead in honey bees was clearly demonstrated by data presented in a 1976 legal suit brought by a Montana beekeeper against a copper smelter. These findings are summarized and discussed in terms of relevance to the Colstrip biomonitoring.

INTRODUCTION

In 1974, I initiated a program utilizing honey bees from commercial apiaries as biomonitors of the bioenvironmental impacts of the coal-fired power plants located at Colstrip, Montana. I hypothesized that honey bees could serve as indicators of pollution impacts: (1) they provide an early warning and a means of continuously monitoring for the presence and amounts of atmospheric pollutants in the environment, and (2) honey bees could be used as environmental bioassays to measure the effects of pollutant mixtures entering ecosystems or to evaluate the suitability of the ambient environment to support bees and other pollinators. Although attempts had been made by investigators both in Europe and the United States to use honey bees in this manner, the Colstrip project was unique. It provided an opportunity to: (1) conduct baseline studies in a relatively pristine area before power plants or other industrial sources of anthropogenic pollutants began operation; (2) monitor post-operational changes in the ecosystems of the Northern Great Plains; and (3) follow (over a six-year period) the effects of long-term exposure to low levels of air pollutants.

Honey bees were chosen for use as indicator species and as monitors for several reasons. They are efficient foragers and gatherers of materials from the environment. They may forage as far as 8 km or more from their hives, canvas several thousand acres and visit innumerable flowers. Bees collect pollen, nectar, honeydew, resin and water. Behaviorally and structurally, honey bees are adapted for the efficient collection of these substances. They seem unable to screen out potentially hazardous substances which settle on plant surfaces or on the bees themselves or which become associated or entrapped in honey, pollen or water. Bees are likely to inhale fine dusts, acid mists or gases since the respiratory passages of bees open directly to the atmosphere and lack protective linings such as mucosa. Hairs surrounding the spiracles filter out larger particles.

Honey bees are known to be magnifiers of many contaminants. In general, levels of air pollutants are highest in the tissues of the oldest adult bees, lower in young bees, and much lower in pollen, nectar, floral parts or water. Since honey bees are social insects, they can be managed. They are a useful indicator organism because they can be transported to monitoring sites, they collect copious quantities of pollen and honey that can be sampled, and there are thousands of bees in a colony. Analysis of vegetation and water samples collected in the vicinity of apiaries and physical and chemical air quality data along with the bee studies will give a comprehensive profile of environmental quality. This comprehensive data reveals the transportation routes of contaminants through honey bee systems. Honey bees should serve as indirect tracers of the passage of pollutants through pollination systems.

Honey bees act as accumulators or concentrators of noxious substances and as such can be used for exposure monitoring. They tend to be more sensitive than many other insects and higher animals such as livestock to harm from these materials. This sensitivity can be used as a leading indicator of harmful effects. These effects are manifested in several ways. For example, air pollutants, pesticides and anesthetics have induced shortened life spans, altered brood laying and rearing, reduced hatchability and fecundity, genetic alterations, toxicant avoidance, disorientation, memory loss, and both temporary and permanent behavior modification. Ultimately, lack of colony vigor and other effects such as reduced pollination, depressed wax and lessened honey production may occur. Thus, not only bees but in turn beekeepers and growers of crops reliant on insect pollination may be affected. More difficult to detect but equally important are impacts to pollination of indigenous vegetation. Many ground cover and soil holding plant species rely on insect pollination. Thus, honey bees offer considerable potential as biological indicators and monitors for environmental assessments and are ecologically and economically important as organisms which need to be protected from significant harm.

There are more than 6,000 colonies located in the Colstrip area. Per colony honey production by these Colstrip bees ranks among the highest in the nation. Colstrip bees also pollinate alfalfa seed crops grown in southeastern Montana. Approximately one-third of these colonies are transported yearly to California for rental as pollinators of almond groves, orchards and truck gardens. Detailed discussions of the effects of air pollutants on honey bees, pertinent agricultural statistics about honey production and the beekeeping industry of Montana, and detailed descriptions of the materials and methods incorporated in this project are presented by Bromenshenk (1976, 1978, 1979).

MATERIALS AND METHODS

In 1978, honey bees, pollen, air and water were sampled from 15 apiaries during late June and again in mid-September. By June, contaminants which may have been carried back with the "migrant" colonies that had been taken to California to be rented for pollination should have been cleared out of the colonies. Tests conducted in 1975 indicated that very little fluoride or arsenic was found in any colonies returned from California. Food supplies brought back from California with the bees were consumed and replenished within a few weeks. By late June, all colonies had had several population turnovers since the return from California in April and early May. Colonies distant from Colstrip are not moved out of the region. As such, these can be used as checks as can be the one to two colonies which were over-wintered at each sampled apiary. September is the latest that most of the apiaries could be sampled because most of the hives were taken back to California in early October.

Each apiary contained at least 15 colonies, some as many as 50, while most had 20 to 30 colonies. At each beeyard, approximately 300 bees (30 gm wet weight) were obtained at the entrance of each sampled hive by using a high velocity, battery-powered, acrylic vacuum apparatus. This drew bees directly into plastic sample jars. All samples were fast frozen in sealed sample jars. The jars containing bees were immediately placed under blocks of dry ice held in heavy-duty cooler chests. Each chest was lined with 5 cm of styrofoam and encased in 1.2 cm thick plywood sheathing coated with fiberglass. A minimum of 20 to 25 kg of dry ice was maintained in the chests. After being frozen, samples were transferred to Whirl Pacs® and stored at -20°C until analyzed. Pollen was obtained by removing pellets from honeycombs using a plastic pick and was stored in plastic vials at room temperature. Water was obtained from the closest supply, frozen immediately in the plastic sample bottles, and stored in a freezer. Sulfation and calcium formate plates were mounted on posts in each apiary in June and collected in September in order to measure ambient air concentrations of reactive sulfurs and fluorides.

Bee and pollen samples were collected from each of ten hives at each location. Also, a pooled sample of approximately 1,000 bees (100 gm wet weight) was obtained by sampling bees at the entrance of every hive in each beeyard. These samples provided a quick screening method of analyses, produced an average sample from each location, and ensured sufficient quantities of materials for tests which required large numbers of bees such as quality assurance tests and determinations for materials such as pesticides.

Pesticide determinations were not carried out in 1977 or 1978 because no appreciable levels of insecticides in honey bees were detected in 1974 and 1975. In the event that an unusual mortality was observed or a report of pesticide usage in the test areas was received, pesticide analyses could be performed. In general, these analyses are time consuming and costly. As such it was decided to conduct them based on a need for the information.

Sulfur levels in honey bees were not examined in 1978. Determinations of the total bee tissue sulfur content were made in 1974 and 1975. In no cases were significant differences in sulfurs in bee tissues discerned, even in bees placed at the EPA sulfur dioxide zonal air pollution system. Relatively high and variable background sulfur levels (3,500 to 4,800 ppm) in bee tissues probably masked any changes attributable to small incremental additions of anthropogenic sulfurs. Hillmann (1972) came to this conclusion when he was unable to show increases of sulfurs in bees fumigated by several thousand parts per million of sulfur dioxide injected into hives.

Based on the 1974 through 1977 results of investigations near Colstrip for 1978, I decided to delete sulfur determinations, to continue to monitor fluorides in honey bees and to include determinations of arsenic. Both fluoride and arsenic are toxic to honey bees and accumulate in colonies. However, sample preparation and analyses procedures differed for these substances.

Fluoride Analyses

Before being analyzed for fluoride, whole bees and pollen were oven-dried at 50°C for seven days and ground in a Wiley-Mill ® to pass a 40-mesh screen. For each sample, 0.5 gm of ground and dried material was placed in a metal crucible and slurried with distilled water with 0.05 gm of reagent grade calcium oxide. Next, each sample was charred under infrared lamps before being ashed in a muffle furnace at 600°C for at least six hours (usually overnight). The ashed samples were digested in 2 ml of perchorlic acid and subsequently diluted to 100 ml total volume with Orion Tisab total ionic strength activity buffer. Fluoride determinations were made using an Orion specific ion probe inserted into the 150 ml beakers containing the dissolved samples which were agitated constantly by a teflon-coated magnetic stirring bar during the fluoride determinations.

Arsenic

All arsenic determinations were made by A. R. Neuman, Research Chemist, Animal and Range Science, Montana State University. Samples were oven-dried in glass beakers covered with watch-glasses to minimize exposure to dust carried on the air currents in the forced air ovens. One gram samples were weighed into 125 ml Erlynmeyer flasks and digested in 30 ml of a 3:2 mixture of nitric perchloric acid. Samples were left overnight and then heated slowly to solubilize. The heat was gradually increased to reduce sample volume to one-half. The samples were cooled and 10 ml of a 1:1 mixture of nitric:sulfuric acid added. Heat was increased until perchlorate fumes evolved out of the flasks. Heating continued until dense white sulfate fumes occurred. Volume was reduced by heating to 5 ml, and the samples allowed to cool. The cooled samples were transferred to 50 ml volumetric flasks containing 15 ml of concentrated hydrochloric acid, 10 ml of water and 1 ml of 1% (w/v) potassium iodide. Cooling to ambient temperature and allowing the samples to stand for one hour allowed reduction of As^{+5} to As^{+3}

Standards were prepared in 100 ml volumetric flasks which contained 50 ml hydrochloric, 2 ml of potassium iodide and 5, 15 and 25 ng of arsenic. These were allowed to stand for one hour prior to analyses.

All determinations were made using an atomic absorption spectrophotometer connected to an arsine generator. Instrument parameters were:

Wavelength: 193.7

Slit Width: 1.0 nm

Hollow Cathode: 7.0 mA
(not to be exceeded)

Mode: Absorbance/Peak

p.m.: 495V with BC6

Atomized: N_2/H_2 7.5 @ 60 psi/3.0
@ 15 psi

The analytical procedure consisted of placing a 20.0 ml aliquot of standard/sample into the reaction flask. The sample was purged by bubbling 50% N_2 through the solution. Five ml of 5% (w/v) sodium borohydrate was added thru the septum while stirring vigorously. Neuman presented the following example:

Standards	Total Arsenic	Absorbance	Absorbance	ng As/g
5 ng As/ml	100 ng (5 ng X 20 ml)	0.060 0.055 0.058	0.058	0.25
15 ng As/ml	300 ng	0.135 0.178 0.180	0.164	0.75
25 ng As/ml	500 ng	0.238 0.250 0.270	0.253	1.25

r^2 value = 0.997

As an additional check on the arsenic determinations and in order to gain an insight into other trace elements which may be found in bee tissues, samples from an apiary located near the EPA ambient air monitoring trailer at Hay Coulee were sent to Ames Laboratory, Iowa State University, for neutron activation determinations. At the time of this report, the samples had been analyzed, but the final concentrations had not been computed.

Basic statistics have been completed for fluorides and arsenic, although additional statistical treatments by a variety of statistical techniques are ongoing. Previous statistical tests (1974 through 1977) have indicated that post-operational fluoride levels in honey bees may not be normally distributed. Therefore, in addition to parametric tests such as analyses of variance which assume normality of distributions (Sokal and Rohlf, 1969), Kruskal-Wallis and Wilcoxon two-sample tests were used. In this case, Kruskal-Wallis tests the null hypothesis that all the populations have the same distribution of fluoride concentrations. The alternative hypothesis is that some populations provide higher distribution values than others. A significant value for the Kruskal-Wallis statistic causes a rejection of the former hypothesis.

Fluoride concentrations in adult worker honey bees from individual hives at given locations were considered to be the basic group for comparisons of pre- and post-operational data. Using a Dec-20 computer, fluoride values for honey bees from all colonies were ranked. First, all variates from all groups were pooled. Then the variates were sorted from lowest to highest (X_0, X_1, X_2 ...X_n). Next, the sorted observations were ranked from low to high (R_1, R_2, R_3 ...R_n), sorted back into the original groups, and average ranks computed as:

$$\frac{\overset{n_i}{(\Sigma R)_i}}{n}$$

These calculations were conducted for all sites and all years (1975 through 1977) and for the principal sites by year (1975, 1977, 1978).

The Wilcoxon two-sample test will be used to determine whether there was a significant difference in distributions of levels of fluoride in or on bees at a given location (apiary). For example, if X designates 1975 values, Y, 1976 values, Z, 1978 values, and E expected, then the null hypotheses may be stated as follows:

A (one-tailed test)	$H_0 : E(Y) \leq E(X)$
	$H_1 : E(Y) > E(X)$
B (one-tailed test)	$H_0 : E(Z) \geq E(Y)$
	$H_1 : E(Z) < E(Y)$

RESULTS

All analyses of bees, pollen and water from 1978 were complete at the time of this report. In addition, a back log of early season analyses for 1977 were finished.

Bees do not forage at random. They display preferences for particular flowers and foraging areas (Doull, 1971). If an area is subjected to airborne pollutants, one would expect that the ambient concentrations of contaminants would be affected by factors such as topography and meteorology. As such, some areas would be exposed to higher concentrations of pollutants. One would expect that bees foraging in regions of greater pollution exposure would contact and bring back more anthropogenic materials than bees foraging in parts receiving less exposure. Bees from a colony may forage up to 8 km, although they usually stay within 2 to 3 km (McGregor, 1976). Because of this, bees from colonies in the same beeyard may gather materials from different places and bring back different quantities of pollutants. Thus, bees from a colony foraging an area of relatively high pollution impact could be expected to demonstrate higher concentrations of contaminants than bees from a colony that foraged in a part of the region exposed to lower impacts. In a pristine area, regardless of where the bees foraged, the relative differences of exposure should be slight, unless there is some localized source of pollutants. Therefore, the levels of noxious substances under these conditions should be essentially the same from colony to colony. Because of preferential foraging and non-uniform exposures under pollution stress, variance of levels of materials such as fluoride in bees should increase as well as mean concentration. For this reason, whereas in 1974 through 1976 four individual colonies were sampled, beginning in 1977 the number of individual colonies was increased to ten. The application of Stein's two-stage sample test to determine the number of observations necessary for a mean with a confidence interval of a prescribed base is discussed by Bromenshenk (1979).

My previous reports (1979) did not include the fluoride analysis results for June, 1977. These results are presented along with a map of the apiary sites in Figure 5.1. Site NE 10 has a water supply containing as much as 20 times the fluoride content of most ground water supplies visited by honey bees. Bees from this apiary have consistently demonstrated high concentrations of fluoride. Baseline levels of fluoride of bees in 1974 and 1975 averaged seven to eight ppm (dry weight). Sites SE 12 and SE 6 are the distant check sites and displayed the lowest fluoride concentrations. Site SE 12 is located 45 km south of SE 6. Sites N 1 and S 1 are closest to Colstrip. Because of an unusually dry summer in 1977, nectar and pollen sources diminished rapidly at many southeastern Montana locations. Colonies from N 1 and S 1 were moved to California in August in order for the bees to replenish marginal food supplies. Thus, there were no beehives at these two locations in September. However, the June 1977 fluoride levels at these sites were the highest observed in southeastern Montana. These levels exceeded baseline by a factor of fourteen. Figure 5.2 typifies baseline levels of fluoride in honey bees and illustrates the biomagnification that occurs.

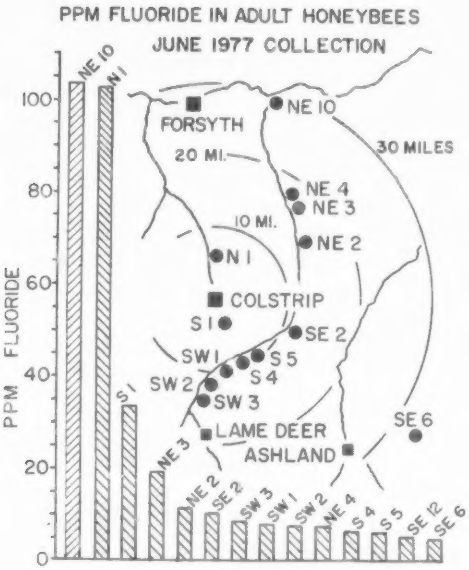

Figure 5.1. Locations of apiaries with respect to Colstrip and June 1977 levels of fluoride.

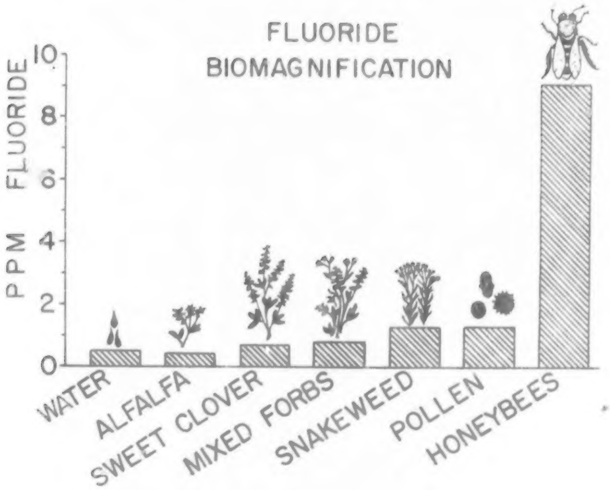

Figure 5.2. Biomagnification of fluoride by honey bees at baseline concentrations (1975 data collected in the Colstrip area).

I have carried out investigations since 1974 at several Montana locations subjected to fluoride from sources such as oil refineries, aluminum reduction facilities and chemical plants. These studies demonstrate that fluoride concentrations in honey bees may be as much as 20 times greater than the baselines established in southeastern Montana. In addition, whenever fluoride levels in or on honey bees exceeded 120 to 130 ppm, beekeepers reported poor production and weak colonies. This is consistent with literature reports concerning toxic doses of fluoride and case history levels of flouride reported for dead bees in industrial zones where severe and sudden bee mortality had occurred. Figure 5.3 summarizes these findings.

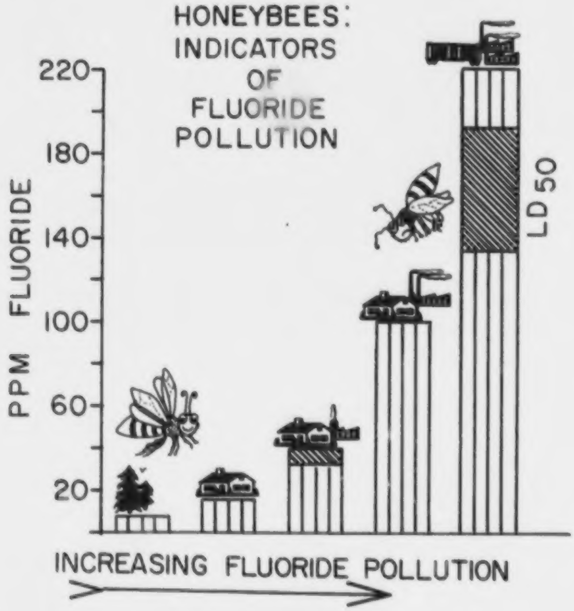

Figure 5.3. Levels of fluorides in bees from pristine and industrial areas. LD_{50} based on literature reports.

The results of the fluoride determinations for adult honey bees collected in southeastern Montana in June and September, 1978, are presented in Table 5.1. Fluoride levels in pollen are given in Table 5.2 and fluoride levels in water presented in Table 5.3. Included in these tables are basic statistics--mean, standard deviation and standard error.

Bees collected in September were exposed throughout the growing season to emissions from the Colstrip power plants. Since many of the colonies near Colstrip are transported to California in autumn and returned in the spring, bees collected in June for the most part were exposed for a period of 60 days or less, compared to an exposure period of approximately 135 days for bees sampled in September. Therefore, the September sampling period should reflect any long-term accumulation trends. Fluoride concentrations in bees in August and September for all years are presented in Figure 5.4.

TABLE 5.1. FLUORIDE CONTENT OF ADULT WORKER HONEY BEES, 1978

Site	Date	1	2	3	4	5	6	7	8	9	10	Combined Sample *	$\bar{x}$	S.D.	S.E.	95% Confidence Interval**
NE 2	June	6.1	3.9	5.4	6.3	5.1	7.3	4.2	6.2	3.6	4.5	5.9	5.3	1.2	0.38	4.4 - 7.5
	Sept	10.1	10.6	11.3	10.7	6.8	13.3	7.1	10.0	5.7	10.3	12.6	9.6	2.3	0.74	7.9 - 11.3
NE 3	June	7.2	7.6	7.0	19.5	11.3	5.2	18.3	11.5	21.6	12.1	10.7	12.1	5.8	1.83	8.0 - 16.2
	Sept	6.4	6.3	6.8	10.6	11.0	8.4	11.3	15.5	8.2	14.0	11.0	9.8	3.2	1.01	7.6 - 12.1
NE 4	June	6.9	6.0	4.6	11.4	3.6	3.4	4.5	4.8	4.3	5.5	4.5	5.5	2.3	0.74	3.8 - 7.2
	Sept	11.1	12.4	8.1	14.6	11.6	6.8	7.4	7.8	11.8	12.8	11.0	10.4	2.7	0.85	8.5 - 12.4
NE 10	June	80.0	65.3	50.2	40.6	37.8	48.4	105.4	105.8	59.1	122.9	73.0	71.6	30.4	9.62	49.8 - 93.3
	Sept	84.9	82.9	56.3	53.5	51.8	44.7	72.1	30.9	86.4	90.5	57.9	65.4	20.6	6.53	50.6 - 80.2
SE 1	June	3.3	5.2	4.6	6.9	5.0	5.8	5.5	4.4	5.3	4.4	4.5	5.0	1.0	0.31	4.3 - 5.7
	Sept	10.4	12.1	7.9	11.7	6.5	11.4	12.2	11.4	9.4	15.0	13.6	10.8	2.4	0.76	9.1 - 12.5
SE 2	June	5.7	5.6	3.5	6.4	4.1	5.0	7.2	8.3	5.8	12.0	6.5	6.4	2.4	0.77	4.6 - 8.1
	Sept	6.7	5.5	5.9	10.8	5.1	8.1	11.3	8.8	12.3	10.2	6.6	8.5	2.6	0.83	6.6 - 10.4
SE-6	June	4.9	2.7	2.6	2.6	5.5	5.4	4.9	5.9	3.2	5.3	4.1	4.3	1.4	0.43	3.3 - 5.3
	Sept	7.6	7.1	8.6	5.8	9.0	7.9	8.8	6.1	9.4	4.3	6.5	7.5	1.6	0.52	6.3 - 8.6
SE-12	June	5.0	5.3	4.8	5.0	5.7	2.7	4.8	5.6	4.0	5.5	4.2	4.8	0.9	0.29	4.2 - 5.5
	Sept	7.5	6.3	7.6	7.1	7.8	6.1	5.7	5.9	7.9	6.1	8.1	6.8	0.9	0.27	6.2 - 7.4
S-1	June	3.7	6.3	3.4	5.2	6.8	3.8	3.9	7.0	4.1	4.7	4.8	4.9	1.4	0.43	3.9 - 5.9
	Sept	8.9	5.0	6.3	4.4	5.1	4.3	4.0	6.1	8.3	5.9	4.9	5.8	1.7	0.53	4.6 - 7.0
S-4	June	2.8	4.3	5.6	5.9	6.6	3.4	7.2	7.0	6.3	6.9	4.1	5.6	1.6	0.50	4.5 - 6.7
	Sept	8.4	6.8	9.2	15.3	12.0	6.8	13.5	13.4	11.9	11.2	12.5	10.9	2.9	0.93	8.8 - 13.0
S-5	June	4.0	4.3	7.2	5.1	5.4	5.0	4.4	3.3	3.5	1.9	4.5	4.4	1.4	0.45	3.4 - 5.4
	Sept	6.9	11.2	10.6	11.7	10.9	12.0	11.3	6.4	10.4	6.5	8.0	9.8	2.2	0.71	8.2 - 11.4
SW-1	June	5.9	5.2	5.4	5.6	7.3	6.9	7.2	6.5	8.2	4.5	6.2	6.3	1.1	0.36	5.5 - 7.1
	Sept	12.4	11.7	12.7	12.0	7.0	12.7	8.0	16.3	8.0	15.5	11.3	11.6	3.1	0.99	9.4 - 13.9
SW-2	June	4.7	4.8	3.9	6.4	3.9	5.7	5.4	3.5	4.6	4.8	6.1	4.8	0.9	0.28	4.1 - 5.4
	Sept	13.6	11.3	11.0	7.3	10.2	11.9	12.5	6.6	11.4	7.6	11.7	10.3	2.4	0.75	8.6 - 12.0
SW-3	June	4.8	4.0	5.1	3.1	3.1	5.1	5.0	5.9	5.1	3.0	4.8	4.4	1.0	0.33	3.7 - 5.2
	Sept	6.3	8.4	7.9	8.6	6.4	8.1	10.3	10.7	6.7	6.9	7.9	8.0	1.5	0.49	6.9 - 9.1
GB-3	Sept	11.4	6.9	10.5	8.5	7.2	7.5	7.7	7.6	11.2	7.4	7.5	8.6	1.8	0.55	7.4 - 9.8

* Each value represents a sample combined in the field from 15-40 colonies.

** $\mu = \bar{x} \pm t_{.05} S_{\bar{x}}$

TABLE 5.2. FLUORIDE IN POLLEN, 1978

Site	$\overline{X}$	S.D.	S.E.	N
NE 2	2.29	1.02	0.323	10
NE 3	2.43	1.40	0.468	9
NE 4	2.01	0.79	0.264	9
NE 10	2.32	1.27	0.424	9
SE 1	2.96	1.07	0.377	9
SE 2	2.65	1.04	0.330	10
SE 16	2.26	1.05	0.348	10
SE 12	2.29	0.80	0.253	10
S 1	2.42	1.55	0.498	10
S 4	1.62	0.77	0.257	9
S 5	2.15	1.48	0.467	10
SW 1	2.55	0.90	0.286	10
SW 2	2.11	1.21	0.382	10
SW 3	1.80	0.66	0.219	9
GB 3	4.20	2.74	0.866	10

TABLE 5.3. FLUORIDE IN APIARY WATER SUPPLIES, 1978

Site	NE 2	NE 3	NE 4	NE 10	SE 1	SE 2	SE 12	S 1	S 4	S 5	SW 1	SW 2	SW 3	GB 3
1977	0.5	0.5	0.5	12.7	0.5	0.6	0.6	0.2	0.5	0.5	0.5	0.5	0.5	0.3
1978	0.5	0.5	0.3	6.8	0.6	0.6	0.5	0.2	0.6	0.6	0.6	0.6	0.6	0.5

All Sites (1977)	All Sites Except NE 10 (1977)	Rosebud Creek Sites (1977)	All Sites (1978)	All Sites Except NE 10 (1978)	Rosebud Creek Sites (1978)
$\overline{X}$ = 1.35 S.D.= 3.27 S.E.= 0.85 N = 14	$\overline{X}$ = 0.48 S.D.= 0.11 S.E.= 0.03 N = 13	$\overline{X}$ = 0.51 S.D.= 0.03 S.E.= 0.01 N = 10	$\overline{X}$ = 0.96 S.D.= 1.68 S.E.= 0.45 N = 14	$\overline{X}$ = 0.52 S.D.= 0.13 S.E.= 0.04 N = 13	$\overline{X}$ = 0.55 S.D.= 0.10 S.E.= 0.03 N = 10

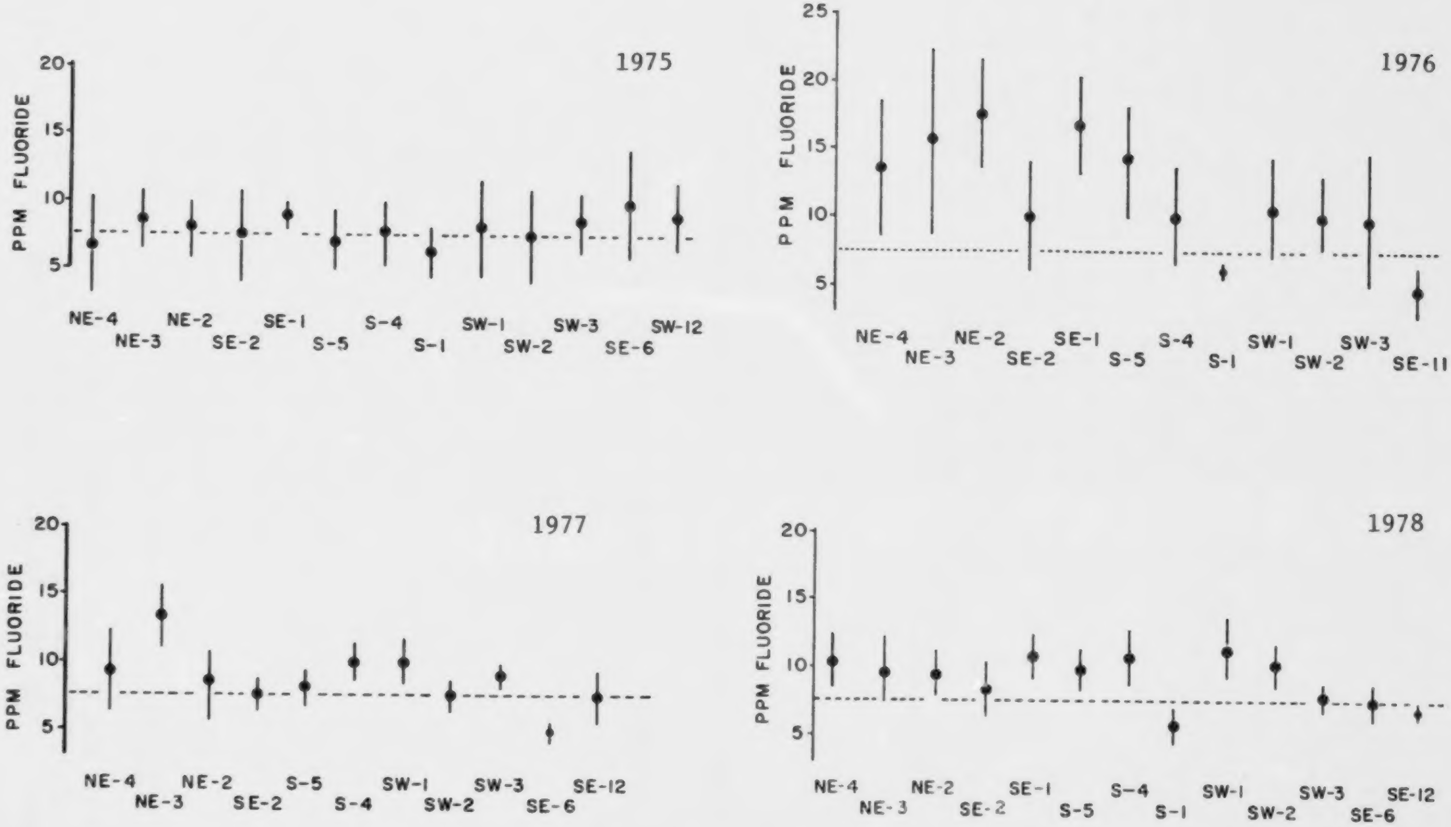

Figure 5.4. Mean fluoride content and 95% confidence intervals of worker honey bees collected in autumn, 1975, 1976, 1977 and 1978.

Fluoride levels were lowest throughout the region in 1975 and demonstrated relatively small differences in mean fluoride. Fluoride levels were highest at check sites 40 and 80 km distant from Colstrip. In 1976, fluoride levels at apiaries within 20 km of Colstrip were highest, mean fluoride at some sites downwind from the power plants were double the 1975 values, and site-to-site variability of means was considerably greater than for 1975. Unlike 1975, fluoride levels at the check sites were lower than at any of the Colstrip sites. In 1977, trends of increased mean fluoride at sites near Colstrip and of considerable site-to-site variability of means continued. However, the levels were not as high as for 1976 and in several instances approximated the 1975 levels. Fluoride levels at the check sites tended to be lower than at most of the sites near Colstrip. Fluoride levels in 1978 were generally increased at most apiaries near Colstrip relative to levels in 1975 and in 1977. None of the 1978 values for specific sites were as high as peak values observed in 1976 and 1977, although many sites demonstrated lower mean values in 1977 than in 1978. As in other post-operational years, values at the check sites were lower than for most of the Colstrip sites. Changes in levels of fluoride in honey bees at sites near Colstrip were significant for 1976 and 1977 and were not normally distributed as compared to the pre-operational levels of 1975 (Bromenshenk, 1978, 1979).

Kruskal-Wallis test results for fluorides in honey bees from 1975 through 1978 are presented in Table 5.4. Data for 1974 through 1977 have been previously published by Bromenshenk, (1979). Based on these results, the null hypothesis was accepted for 1975. Pre-operational fluoride distributions were the same at all locations. For 1977 and 1978 (post-operational), honey bees displayed a higher distribution of fluoride concentrations than during 1975.

TABLE 5.4. KRUSKAL-WALLIS TEST OF FLUORIDE IN ADULT WORKER HONEY BEES

Site	Year	Kruskal-Wallis Statistic, H	df	Critical X^2	P
All Sites (n = 48)	1975-77	151.50	47	63.98	$p \leq 0.005$
Principal Sites (n = 14)	1975	21.78	13	22.36	n.s.
	1977	54.74	13	22.36	$p \leq 0.005$
	1978	47.58	12	21.03	$p \leq 0.005$

Results of the Wilcoxon two-sample tests of year-to-year differences in the distributions of fluoride levels in bees at each apiary were not available for this report. They will be included in the Sixth Interim manuscript.

Fluorides in Pupae Versus Adults, Soft Tissues Versus Exoskeleton

In September, 1978, pupae were obtained from three of the sampled hives at five locations. A section of comb containing 105 to 200 pupae was removed from each colony at the time that the adults were vacuumed from the entrances. Bees at site NE 10 were exposed to fluoride in the water supply. Site GB 3 is located in Billings and has demonstrated at times levels of fluoride in adults of 30 to 40 ppm, presumably related to fluoride in the ambient air from nearby oil refineries. Sites S 5 and SE 1 are near Colstrip. Site SE 12 is from a check yard near Fort Howes, Montana. The pupae were extracted from the comb in the laboratory using stainless steel forceps (Figure 5.5). The results are given in Table 5.5.

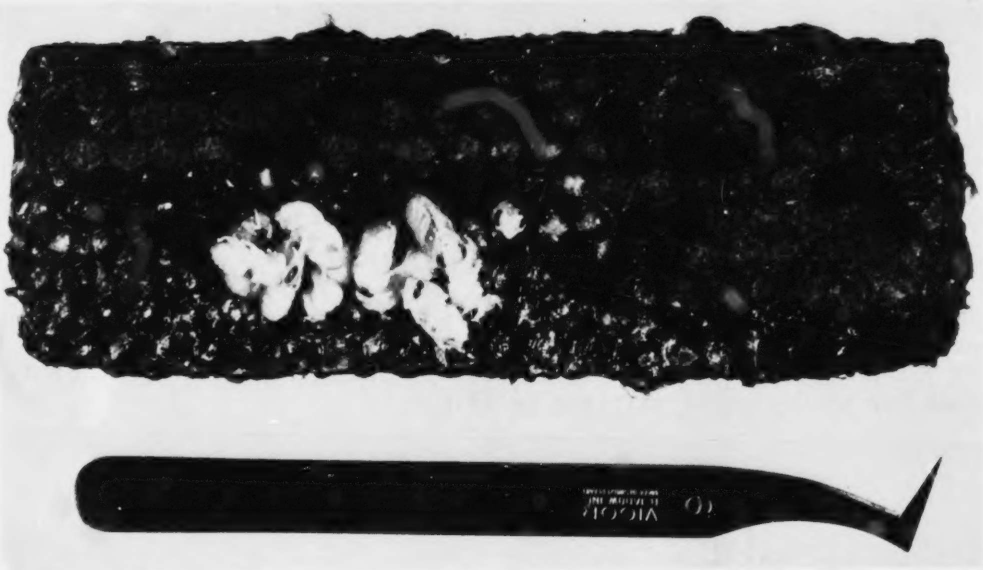

Figure 5.5. Honey bee pupae extracted from comb. Capped cells still contain pupae.

Although there was slightly more fluoride in pupae from colonies using the fluoride-contaminated water supply than in pupae from the other apiaries, it is clear that relatively little fluoride reaches the pupae even when adult bees demonstrate relatively high fluoride concentrations. Levels observed in pupae were one-third to one-half that in adults from pristine areas. Levels of fluoride in pupae were similar to levels of fluoride in pollen from the same colonies.

Preliminary results indicate that in bees exposed to fluoride in water (NE 10) and in bees where water was a suspected source (N 1), levels of fluoride in the soft internal tissues versus the hard exoskeleton were essentially the same. Three hundred bees were dissected for each test. Bees from N 1 contained

40.1 ppm fluoride in the exoskeleton and 40.7 ppm in the internal tissues. Bees from NE 10 contained 120.3 ppm of fluoride in the exoskeleton, 140.9 in the internal tissues.

TABLE 5.5. FLUORIDE IN PUPAE VERSUS ADULT WORKER HONEY BEES

Site	Hive No.	Fluoride - Pupae		Fluoride - Adults	
GB-3	1	1.8	$\bar{X} = 1.47$	11.4	$\bar{X} = 9.10$
	4	1.6	$S = 0.42$	8.5	$S = 2.07$
	10	1.0		7.4	
S-5	1	0.3	$\bar{X} = 0.60$	6.9	$\bar{X} = 5.67$
	4	0.5	$S = 0.36$	5.6	$S = 1.20$
	10	1.0		4.5	
SE-1	1	1.0	$\bar{X} = 1.60$	10.4	$\bar{X} = 12.37$
	4	2.4	$S = 0.72$	11.7	$S = 2.37$
	10	1.4		15.0	
SE-12	1	0.8	$\bar{X} = 1.50$	7.5	$\bar{X} = 6.90$
	4	2.0	$S = 0.62$	7.1	$S = 0.72$
	10	1.7		6.1	
NE-10	1	2.8	$\bar{X} = 2.20$	84.9	$\bar{X} = 76.30$
	4	1.4	$S = 0.72$	53.5	$S = 19.94$
	10	2.4		90.5	

Pooled Sample versus Observations from Individual Hives

Figure 5.6 shows the fluoride content determined by analyses of pooled samples versus the mean fluoride obtained from ten independent observations. Table 5.1 presents mean fluoride levels and fluoride content of pooled samples for all apiaries utilized in 1978.

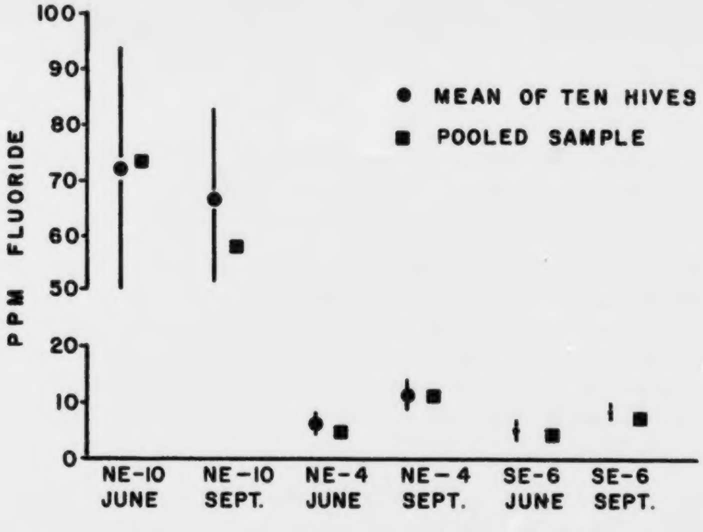

Figure 5.6. Fluoride content of worker honey bees.

Arsenic and Trace Metals

The results of the atomic absorption spectrophotometry analyses for arsenic in or on adult worker honey bees sampled near Colstrip are listed in Table 5.6. Mean arsenic in bees ranged from 0.37 to 0.55 ppm. Arsenic concentrations of pooled samples for 1974, 1975, 1976 and 1977 were respectively 0.41 ± 0.25 (1 standard deviation), 0.36±0.13; 0.49±0.16; and 0.57±0.27 ppm. Minimum and maximum individual values ranged from 0.1 to 1.3 ppm. The 1.3 was observed at a site northeast of Colstrip in 1977 and was the highest value recorded at any site for any year in southeastern Montana. The next highest value value was 0.8. If the 1.3 value is excluded from the 1977 data as an outlier, the 1977 mean for the yards sampled becomes 0.50 ± 0.13. For the most part, these samples represent pooled samples from the apiaries obtained in the autumn of each year, although the 1975 values are based on collections made throughout the growing season. For comparison, samples obtained near Butte from areas subjected to arsenic impacts from a copper smelter demonstrated levels of arsenic ranging from 1.2 to 3.06 ppm (1975). Similarly, a bee sample taken from a site near an aluminum reduction facility at Columbia Falls, Montana had 2.03 ppm arsenic (1975).

TABLE 5.6. ARSENIC CONTENT (PPM) OF HONEY BEES FROM SOUTHEASTERN MONTANA

YEAR	N	$\bar{X}$	S.D.	S.E.
1974	10	0.41	0.25	0.079
1975	10	0.36	0.13	0.041
1976	8	0.49	0.16	0.057
1977	11	0.57	0.27	0.081
1977*	10	0.50	0.13	0.039
1975**	4	0.55	0.17	0.085
	4	0.38	0.15	0.075
	4	0.25	0.10	0.050

* 1.3 ppm value excluded as an outlier.
** Each set represents values from four individual hives at one site.

A legal suit brought by a Montana beekeeper against a copper smelter alleging arsenic poisoning of over 400 colonies of honey bees included among the exhigits extensive tables of results of analyses of heavy metals in bees. (Furlong *et al.* correspondence, 1973; 1975; 1976). The data covers lead, zinc, copper, cadmium, arsenic and fluoride in bees from the Deer Lodge Valley during 1973 through 1976. For the most part, analyses were performed via atomic absorption spectrophotometry. Generally, the bees were washed prior to analysis. Based on a test of metal content of bees "as received" versus bee washed with water, the conclusion was drawn that slightly less arsenic was found in the washed bees, but essentially the same concentration of other metals occurred in the unwashed and washed bees. Figures 5.7 to 5.9 summarize data obtained from these exhibits. Seasonal trends of accumulation of arsenic, fluoride and other metals at Deer Lodge are pertinent to my Colstrip observations.

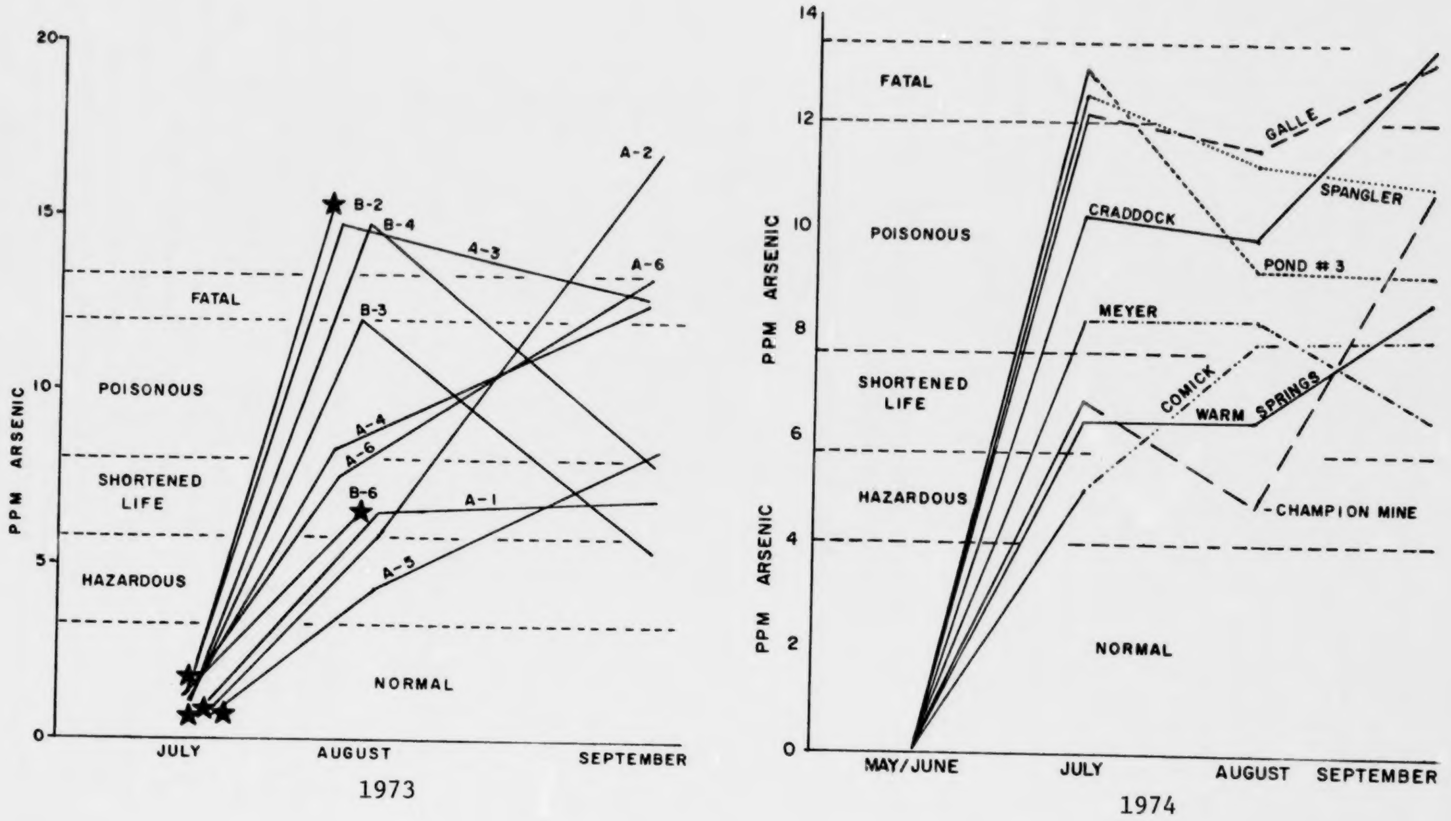

Figure 5.7. Arsenic in adult honey bees, Deer Lodge study, 1973-74. Stars indicate loss of colony being sampled.

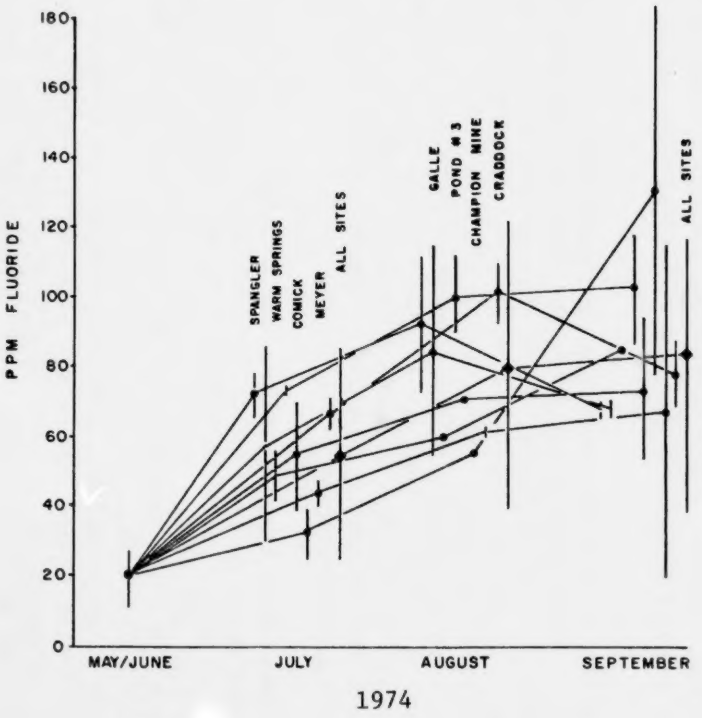

Figure 5.8. Fluoride in adult honey bees, Deer Lodge study.

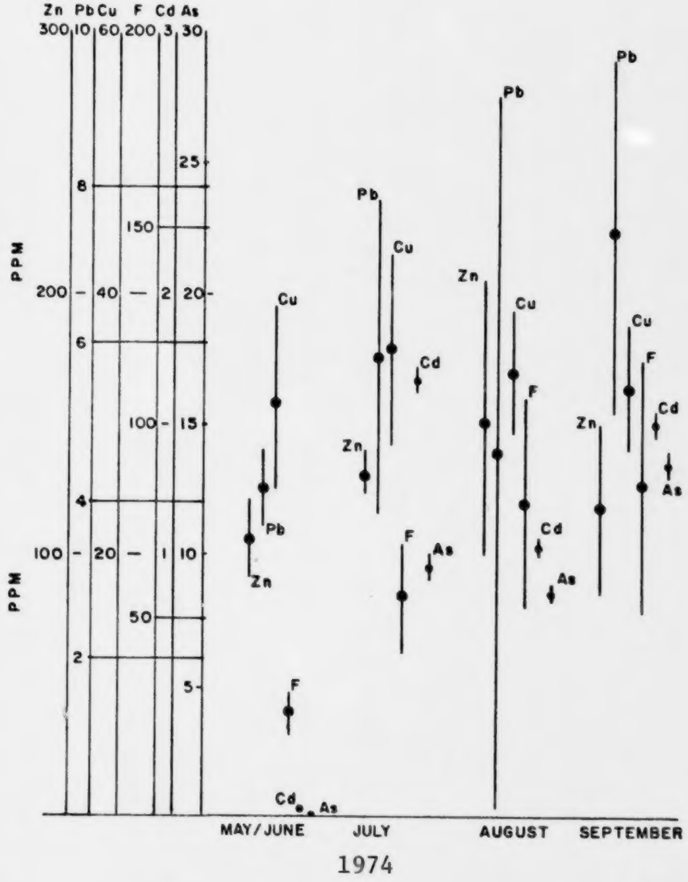

Figure 5.9. Heavy metal content of adult honey bees, Deer Lodge study.

The Deer Lodge study for 1973 and 1974 began each year with package bees obtained from the southern United States. Samples of bees from the packages were analyzed at the start of the tests, although it is not clear in the reports which packages went to which beeyards. Mean values of arsenic, fluoride and cadmium were much lower in the package bees than in any of the later season samples. Levels of zinc and copper in bees sampled in autumn of 1974 were similar to the levels in the package bees, while lead values became highly variable. Arsenic and fluoride levels by July of 1974 had increased dramatically compared to those of May/June. After July, concentrations leveled off, continued to increase or declined somewhat depending on the site. Arsenic concentrations in 1973 displayed similar patterns. In 1975, the fluoride content of bees distinctly increased at only one location. At the other sites late season levels were about the same or lower than the early season values.

Fluoride concentrations in bees from this area were substantially higher than those from southeastern Montana with the exception of the southeastern apiary exposed to fluoride in its water supply. Arsenic concentrations were much higher in bees from the Deer Lodge Valley. One of the highest values observed in the Deer Lodge bees was 36.3 ppm (dry weight) which was 27 times greater than the highest level (1.3 ppm) found in Colstrip bees and 66 to 98 times the mean arsenic content of Colstrip bees.

DISCUSSION

Honey bees as biological tools for environmental assessments of air pollutants are among several methods being developed under the coal-fired power plant project. The ultimate objective of these studies is to provide a means of assessing, predicting and mitigating ecological and related socioeconomic impacts of siting coal-burning electric generators in the Northern Great Plains.

Sulfur dioxide has been the anthropogenic substance of primary interest to the overall coal-fired power plant project. However, as mentioned earlier, honey bees do not appear to be useful organisms for use in exposure monitoring of sulfur dioxide. If the analytical procedures were modified such as utilizing stable sulfur isotopes rather than total sulfurs, it might be possible to distinguish anthropogenic sulfurs in bee tissues from the naturally occurring sulfurs that occur in animal proteins. In other words, my inability to detect incremental increases of sulfurs in bees exposed to sulfur dioxide and other sulfur compounds in the ambient air may be a function of analytical technique rather than of non-accumulation in bees.

It is well known that bees accumulate and concentrate fluoride and arsenic. These materials are toxic to bees. Analyses of stack fly ash from Colstrip Unit 2 indicate that small particles emitted by the stack were enriched in several elements including fluoride and arsenic. Measured stack concentrations of fluoride were 2,130 ± 400 (SD) ppm and of arsenic were 221 ± 20 (SD) ppm (Crecelius *et al.*, 1978). In addition, Munshower (personal communication) detected increased post-operational levels of arsenic in sheaths of pine needles from trees near Colstrip, while investigators at this laboratory have shown post-operational increases in fluoride in pine foliage and in mice sampled near Colstrip. At the time of this report, wind data for 1978 had not been obtained for the Colstrip area. Data from 1976 and 1977 indicated that in the summer winds most frequently blew from Colstrip towards the southeast and northeast. Also, plume strikes have been measured several times a week at an EPA monitoring station at Hay Coulee (See Section 1). This monitor is approximately 11 km southeast of Colstrip, less than 0.5 km from the S 5 apiary location. There were no air monitoring stations near the apiaries northeast of Colstrip. Thus, plume strike data was not available for that vicinity. However, I frequently have observed the plumes from the power plants descending near the ground and traveling relatively intact for at least 10 km, following drainage routes which lead towards the NE 2, 3 and 4 beeyards. I intend to attempt to correlate levels of fluorides in bees with concentrations in the ambient air. Sulfation and calcium formate plates were set out at each of the beeyards during 1978 and 1979. The plates have been sent to the Montana Department of Health for analyses, but the results were not available for this report.

In June, 1977, at N 1, levels of fluoride in bees which exceeded baselines by fourteen-fold were unexpected and puzzling. Although it is possible that the fluoride was carried over from California, this is unlikely. The bees had been at N 1 for several weeks. No other concentrations of fluoride of this magnitude appeared in any other hives sampled. All of the migratory colonies are moved on large flatbed trailers. Colonies are not kept separate on the truck by apiary location. As such, hives marked during the previous summer usually show up at Colstrip distributed in a more or less random pattern across many locations. Therefore, even if the colonies at N 1 had been kept at a site exposed to a source of fluoride before being set in at Colstrip, one would expect to find some of these colonies at other locations or to find some low-fluoride content colonies at N 1.

Air diffusion models predicted that N 1 would be subjected to plume strikes during the winter months, but concentrations of fluoride in bees at N 1 were as high or higher than fluorides in bees sampled in Montana near aluminum reduction facilities and chemical plants. These facilities emit much more fluoride than the Colstrip power plants. Thus, while it is possible, it seems unlikely that this much fluoride would have reached the bees at N 1 via the air. An unanticipated potential fluoride source was discovered in 1978. The ranch owner complained at a legal hearing that hay meadows adjacent to this apiary were being destroyed by waste waters which were being pumped from the coal strip mines into Arnell's Creek. When I visited this apiary in 1978, large areas of the creek bank had been eroded away and cattails were growing in parts of the meadow which previously had supported hay forage. Arnell's Creek typically is dry except during the spring runoff. Water in Arnell's Creek was sampled in 1979 as were bees at N 1. I am trying to obtain 1977 records of chemical analyses of the mine waste water, if any were performed.

Fluorides may concentrate in bees until toxic thresholds are exceeded. The LD_{50} threshold depicted in Figure 5.3, which displays levels of fluorides found in bees from pristine and industrialized zones, is based on literature reviews (Bromenshenk, 1978) and on personal observations made in Montana. The LD_{50} zone is approximate and based on acute toxicities. Apparently no serious attempt has been made to discern what tissue residues are indicative of chronic poisoning and sublethal effects such as shortened life spans, immunological suppression or disruptions of foraging activities.

Elevated fluoride content in bees sampled after the Colstrip power plants began operation as compared to baselines and checks have consistently been observed at apiaries southeast and northeast of Colstrip. In 1978, bees from sites near Colstrip continued to demonstrate significantly higher distributions of fluoride concentrations than baselines. In general, mean fluoride content was higher in Colstrip bees in 1978 than in 1977 and considerably higher than baseline levels, although concentrations were not as high at individual apiaries as in 1976. In 1977, forage was unusually poor because of dry weather, and winds were more variable than in 1976. Based on data available at the time of this report, 1978 was a more typical year than 1977 in terms of moisture, forage and honey production. In all post-operational years, the content of fluoride of bees at distant check points remained at or below baseline concentrations. Fluoride concentrations of surface waters have remained relatively constant for all years. Also, concentrations of fluoride in pollen are essentially the same for all years. Measurements of fluoride and sulfur in the ambient air should provide valuable insights as to the route by which the bees are obtaining these contaminants. This information should be available for the final report.

In 1979 throughout the region, the honey crop was exceptional due to heavy flowering of yellow sweet clover. Therefore, foraging activity should have been great. This should ensure that the bees came into contact with any anthropogenic materials in their surroundings. Since the honey crop was poor in 1977, it will be informative to see the opposite situation.

Fluoride in pupae versus adults clearly demonstrate that pupae are not accumulating fluoride. Either pupae are not being exposed to fluoride or they somehow eliminate it. The former seems probable. In previous studies, foraging adults were found to demonstrate almost twice as much fluoride as hive bees (nurse bees, guards) (Bromenshenk, 1978). The latter probably are only exposed to materials brought back by other bees or to substances that directly enter the hive such as gases. By merit of extensive foraging and by being older, foragers are exposed to more materials and for longer periods than hive bees. If fluoride enters a colony via the pollen, the pupae would be directly exposed since pollen is a major component of the diet of the brood. Fluoride detected in pupae in 1978 tended to be equivalent to levels in pollen, but levels in all pollen samples were low compared to levels in the adult bees and in general not significantly different from site to site. Fluoride in pollen fed to the pupae may be the source of low background levels in pupae. It does not appear to be the source of elevated fluoride in adults. Although water definitely appeared to be the source of high concentrations of fluoride in bees at NE 10 and although bees gather water for diluting honey, for cooling hives and for drinking, fluoride in the water did not appear to affect the fluoride content of the brood. Dilution of food and evaporative cooling of hives by spreading water across the combs seem to be likely routes by which fluoride in water would be transported to the developing brood, but apparently this is not the case. Thus, fluoride in water does not appear to constitute a problem to the brood welfare, although levels in adults approached those known to be definitely toxic. Over the last five years, bees at NE 10 have consistently displayed high body content of fluoride and always have displayed a lack of vigor, slow growth and moderate to low honey production (about 40 pounds per colony). In 1978, the bees were moved to a position on the bank of a creek that averages 0.4 to 0.6 ppm fluoride compared to the 11 to 20 ppm of the stock tank that the bees had previously visited. In 1978, after being moved, colonies at NE 10 made a dramatic turn-around, producing well over 100 pounds per colony (G. Simpson, Beekeeper, personal communication, 1979). They did not display any lack of vigor or other symptoms of chronic fluoride poisoning

Preliminary results of levels of fluoride in or on soft versus hard body tissues demonstrated that the fluoride was not just clinging to the surface such as on the numerous branched hairs that cover the body of a bee. This is consistent with previous observations that washing bees with distilled water prior to analyses did not produce any significant change in the fluoride values (Bromenshenk, 1978). There needs to be more study on fluoride effects and transport through the tissues of bees.

Results of fluoride determinations made for pooled bee samples versus independent samples at each apiary indicate that pooled samples could be utilized in a rapid screening program but do not provide the amount of information, sensitivity nor interpretability of values obtained from many separate colonies.

Observation of 1.3 ppm of arsenic in bees at NE 2 in 1977 raises a warning. Arsenic is released by the Colstrip power plants. It appears to be accumulating in the sheaths of pine needles, it occurred at an apiary that displayed significant post-operational increases of fluoride and it is very toxic to bees. Additional arsenic determinations will be carried out on 1979, 1978 and 1977 samples in order to determine whether this observation was an anomaly or whether arsenic is beginning to reach the bees.

Bees in the Deer Lodge Valley are accumulating and concentrating considerably more arsenic than any bees at Colstrip. This clearly indicates that bees are excellent concentrators of arsenic as well as fluoride. Also, the Deer Lodge data demonstrates that honey bees accumulate and concentrate other metals such as cadmium. This arsenic in bees appears to take its toll. Dashed lines on the graphs of arsenic accumulation in Deer Lodge bees (Figures 5.7 through 5.9) indicate levels reported from case histories near smelters in the United States and Europe to be "fatal" or satisfactory proof of arsenic poisoning" (Knowlton *et al.*, 1950; Debackere, 1972). Note that in most cases, if arsenic in the bees exceeded these thresholds, the bees either were reported to have been lost or in the next sample period showed a lower arsenic concentration. Following this decrease, arsenic levels often increased. This suggests total death of colonies when toxic thresholds were surpassed. In less severe cases, the oldest foragers may have reached toxic levels before younger bees and brood. If the older bees died, this would leave younger bees which should display lower levels of arsenic. As the average age of bees in the colony again increased, longer exposure periods and the shifts to foraging activities should result in increased arsenic content. Admittedly, the forgoing basically is conjecture. However, in 1973 the Montana State Apiarist was called in to examine Deer Lodge colonies because of a suspected pesticide kill. He concluded that the colonies did not exhibit symptoms of poisoning by pesticides. They did appear to be suffering from poisoning by a slower acting toxin such as in air pollutant (W. Kissinger, personal communication, 1979).

An apiary near a copper smelter sustained severe losses of bees near Whitehall, Montana, in 1975. These colonies demonstrated lower levels of arsenic in bees and pollen than Deer Lodge bees but considerably more arsenic than any bees from Colstrip. This illustrates a need for more and better information about arsenic in honey bee systems both as regards the use of honey bees as biological monitors and hazards to bees and the beekeeping industry.

CONCLUSIONS

Honey bees from southeastern Montana continued in 1978 to demonstrate significant post-operational changes in fluoride content compared to 1975 (pre-operational) levels. Extremely high levels of fluoride seen in adult bees at an apiary north of Colstrip in June of 1977 may have been the result of dumping of mine waste waters. Arsenic was found at a considerably higher level in bees at one apiary (NE 2) in September of 1977 compared to levels for all other apiaries and all other years. The arsenic appeared at an apiary that has often demonstrated significant post-operational increases of fluoride in worker honey bees. Preliminary tests indicate that fluoride content of soft and hard body tissues of bees is essentially the same--the fluoride is distributed throughout the body and does not occur just on the body surface. On the other hand, pupae demonstrate relatively low tissue content of fluoride even in instances where the adult bees from the same colonies demonstrate concentrations five to ten times that of baseline values. Also, although relatively high levels of fluoride in water are reflected by high levels of fluoride in adult bees (both in soft and hard tissues), the fluoride does not appear to be affecting or reaching the brood.

Arsenic, as revealed by data obtained from studies in the Deer Lodge Valley, may concentrate in adult bees to levels as great as approximately 100 times that of the levels seen in bees sampled near Colstrip. These levels appear to be harmful to bees causing problems ranging from poor honey production and an inability to overwinter to complete death of the colony. Based on these studies, other materials such as cadmium also are concentrated by bees.

Thus, the results to date reaffirm the belief that honey bees are excellent detectors of contaminants in their surroundings and point to hazards if these materials reach toxic thresholds--problems not only to the bees themselves but also to beekeepers, pollination systems and agriculture.

REFERENCES

Bromenshenk, J. J. 1976. Investigations of the Effects of Coal-Fired Power Plant Emissions Upon Insects, Report of Progress. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana, R. A. Lewis, N. R. Glass, and A. S. Lefohn, eds. EPA-600/3-76-013, U. S. Environmental Protection Agency, Corvallis, Or. pp. 112-129 and 286-312.

Bromenshenk, J. J. 1978. Investigations of the Impact of Coal-Fired Power Plant Emissions Upon Insects. I. Entomological Studies in the Vicinity of Colstrip, Montana. II. Entomological Studies at the Zonal Air Pollution

System. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U. S. Environmental Protection Agency, Corvallis, Or. pp. 146-312 and 473-507.

Bromenshenk, J. J. 1979. Honey Bees and Other Insects as Indicators of Pollution Impact from the Colstrip Power Plants. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 215-239.

Crecelius, E. A., L. A. Rancitelli, and S. Garcia. 1978. Power Plant Emissions and Air Quality. In: Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains and the Consequent Uptake and Turnover in Range Ecosystems, ERDA Annual Report. Activity RX-02-03, Ames Laboratory, Iowa State University, Ames, Iowa. pp. 9-33.

Debackere, M. 1972. Industriele Luchtvervuiling en Bijenteelt (Industrial Pollution and Apiculture). Vlaams Imkersblad., 2(6):145-155.

Doull, K. M. 1971. An Analysis of Bee Behavior as it Relates to Pollination. Am. Bee J., 111(7):266-273; (8):302-303; (9):340-341.

Furlong T., R. Anderson, W. Unger, F. J. Laird Jr., and M. W. Bowman. 1973; 1975; 1976. Correspondence on file at the University of Montana, Missoula, Montana.

Hillmann, R. C. 1972. Biological Effects of Air Pollution on Insects, Emphasizing the Reactions of the Honey Bee (*Apis mellifera* L.) to Sulfur Dioxide. Ph.D. Thesis, The Pennsylvania State University, University Park, Pa. 159 pp.

Knowlton, G. F., A. P. Sturtevant, and C. J. Sorenson. 1950. Adult Honey Bee Losses in Utah Related to Arsenic Poisoning. Utah Agric. Exptl. Stat. Bull. 340. 16 pp.

McGregor, S. E. 1976. Insect Pollination of Cultivated Crop Plants. Agr. Handbook No. 496. USDA Agricultural Research Service, Washington, D.C. 411 pp.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, San Francisco, Ca. 776 pp.

SECTION 6

TEMPORAL VARIATION IN SO_2 CONCENTRATIONS ON ZAPS DURING THE 1978 FIELD SEASON

E.M. Preston, T.L. Gullett, and D.B. Weber

ABSTRACT

Sulfur dioxide exposure regimes on the ZAPS plots during 1978 are characterized. Seasonal mean concentrations are similar to those of previous years on CONTROL, LOW, and MEDIUM plots but were somewhat higher on the HIGH plot. Frequencies of higher concentrations were somewhat greater on most plots than in previous years. The diel pattern of SO_2 concentrations may resemble that of area sources. It does not resemble the point source induced diel cycle near Colstrip. Daytime geometric mean concentrations are substantially less than nighttime concentrations. Diel activity cycles of organisms on the plots must be considered in defining dose-response relationships.

INTRODUCTION

The Zonal Air Pollution System (ZAPS) was designed to allow experimental evaluation of the effects of long-term chronic sulfur dioxide exposure on native grasslands of the northern Great Plains. Experiments are being conducted within two 27-acre grassland exclosures in the Custer National Forest in southeastern Montana. Livestock are excluded to protect them from injury and to protect equipment from damage. Sulfur dioxide (SO_2) fumigation within the first exclosure was initiated in May 1975 (ZAPS I). Fumigation within the second exclosure was started in April 1976 (ZAPS II).

The experiments are designed to test the effects of SO_2 upon biomass dynamics (plant, arthropod, and small mammals), plant and animal community structure, insect and fungal diseases of plants, pollination systems, lichens, and upon a number of physiological and biochemical functions. Dominant plants on the study plots are western wheatgrass (*Agropyron smithii*), prairie junegrass (*Koeleria cristata*), and Sandberg bluegrass (*Poa secunda*).

The design of the ZAPS has been described in detail elsewhere (Lee *et al.*, 1976; Lee and Lewis, 1978; Lee *et al.*, 1978; Lee *et al.*, 1979) and will only be reviewed briefly here. The ZAPS is a network of 2.54 cm diameter aluminum pipes suspended about 75 cm above 0.5 hectare plots of native grassland. Gas release ports (0.8 mm diameter) are positioned at 3 m intervals. There are more than 250 ports per 0.5 hectare plot with no location more than 5.5 m from a port.

Each ZAPS consists of four plots, each receiving different ("CONTROL," LOW, MEDIUM, and HIGH) median concentrations of SO_2. By convention, plots designated A,B,C,D refer to "CONTROL", LOW, MEDIUM, and HIGH SO_2 treatment levels, respectively. The "CONTROL" plot receives no SO_2 from its pipe network, but does receive SO_2 drifting from upwind plots. Consequently, this plot is, in effect, a very low level SO_2 treatment plot whose exposure regime simulates more closely that from a point source than that from an area source.

Exposures are continuous throughout the growing season (April-October). SO_2 is released at constant rates, but ambient concentrations vary with wind dilution and other micrometerological conditions.

In this section, we characterize temporal variation in SO_2 concentration on the ZAPS plots during 1978.

MATERIALS AND METHODS

Sulfur dioxide concentrations were continually measured at a common central point on all plots (location c, see Lee *et al.*, 1979, Figure 1) throughout most of the growing season. Ambient air samples were taken 35 cm above the ground (approximate canopy height).

In general, the procedures described in Lee *et al.* (1979) were followed during 1978. Several minor changes were implemented in order to minimize sources of uncertainty in the SO_2 measurements discussed by Lee *et al.* (1979). First, teflon sample lines were made equal in length (500 m). This minimized differences in response time and resistance to sample flow between lines. Secondly, all dilution air for calibration gas was drawn from outside the monitoring shed and filtered through charcoal before it was used as a carrier for calibration gas. This eliminated CO_2 interference during calibration (Lee *et al.*, 1979).

As in 1977 (Lee *et al.*, 1979), calibration curves relating analyzer response to the logarithms of SO_2 concentration typically became non-linear at SO_2 concentrations below roughly 2 pphm. Linear extrapolation leads to an overestimate of SO_2 levels in the 0-2 pphm range. The true value is likely to lie between .1 pphm and the value estimated from the calibration curve. These values were used to specify upper and lower bounds for any SO_2 concentration falling in the non-linear portion of the calibration curve. During data analysis, each analyzer response was entered into two calibration equations. In one, the HIGH run, the calibration equation was adjusted to yield a maximum value that could result from the above sources of uncertainty. In the other, the LOW run, the calibration equation was adjusted to yield the corresponding minimum estimate. Summary statistics were computed for both the HIGH run and the LOW run.

RESULTS

Interseasonal Trends

Seasonal summaries are given in Tables 6.1 and 6.2. The first number for each entry is the LOW run value, the second is the HIGH run value. If only one number is presented, there was no difference between the LOW run and HIGH run values.

Geometric mean concentrations were similar to previous years for Control, Low and Medium plots. They were somewhat higher for HIGH plots. However, the frequencies of higher concentrations were greater than in previous years on most plots (Lee *et al.*, 1979, Lee *et al.*, 1978). This is reflected in higher arithmetic averages and in larger standard geometric deviations.

Diel Patterns

As in previous years (Lee *et al.*, 1979) SO_2 concentrations on the plots were higher and more variable during the nighttime hours than during daylight hours (Table 6.3). This is caused by generally poor mixing and low wind speeds at night and relatively better mixing and higher wind speeds during the daytime. The diel pattern of SO_2 concentrations is strongly correlated with the reciprocal of wind speed (Figure 6.1). This relationship suggests that wind speeds much above 8 mph reduce geometric mean SO_2 concentrations to background on ZAPS II, HIGH plot. Frequency distributions of SO_2 concentrations for day hours and night hours are compared in Figures 6.2 and 6.3. The combined frequency distributions are also provided since this format has been used in previous interim reports describing the 1975-1977 data. For ZAPS II, concentration frequency distributions are compared with that for Hay Coulee (Ludwick *et al.*, 1979) in Figure 6.4. Hay Coulee is located 7.5 miles southeast of the coal-fired generating units at Colstrip.

Intraseasonal Trends

Trends in SO_2 exposure concentrations over the 1978 season are illustrated in Figure 6.5. On most plots, concentrations increased as the season progressed. This trend was more evident on ZAPS II than on ZAPS I.

DISCUSSION

Biological Significance of the Diel Patterns of SO_2 Exposure on ZAPS

There is a need to convey a general feeling for the SO_2 exposure regime that organisms on the ZAPS plots experience. In previous interim reports seasonal means, seasonal frequency distributions, and aspects of intraseasonal

TABLE 6.1 ZAPS I SEASONAL SUMMARY, 1978*

Control	Geometric Mean = 1.0-1.1	Standard Geometric Deviation = 1.7, 2.1	Arithmetic Mean = 1.4
	1-Hour Ave Exceeded 25 pphm	1 Times:	0.0 Percent
	3-Hour Ave Exceeded 50 pphm	0 Times:	0.0 Percent
	24-Hour Ave Exceeded 10 pphm	0 Times:	0.0 Percent
	24-Hour Ave Exceeded 14 pphm	0 Times:	0.0 Percent
	1-Hour Peak 26	3-Hour Peak 11	24-Hour Peak 5
Low	GM = 2.8	SGD = 2.7	Arithmetic Mean = 5.4
	1-Hour Ave Exceeded 25 pphm	125-127 Times:	3.7 Percent
	3-Hour Ave Exceeded 50 pphm	3 Times:	0.3 Percent(b)
	24-Hour Ave Exceeded 10 pphm	19-20 Times:	12.5-13.2 Percent(a)
	24-Hour Ave Exceeded 14 pphm	3-5 Times:	2.0-3.3 Percent(b)
	1-Hour Peak 110	3-Hour Peak 70	24-Hour Peak 17
Medium	GM = 5.1	SGD = 3.1	Arithmetic Mean = 11.6
	1-Hour Ave Exceeded 25 pphm	426-433 Times:	12.5-12.6 Percent(a)
	3-Hour Ave Exceeded 50 pphm	25-26 Times:	2.2 Percent(b)
	24-Hour Ave Exceeded 10 pphm	76-77 Times:	50.0-50.6 Percent(a)
	24-Hour Ave Exceeded 14 pphm	38 Times:	25.0 Percent(b)
	1-Hour Peak 171-172	3-Hour Peak 109	24-Hour Peak 27-31
High	GM = 8.6-8.9	SGD = 3.2,3.4	Arithmetic Mean = 19.2
	1-Hour Ave Exceeded 25 pphm	728-734 Times:	21.3-21.5 Percent(a)
	3-Hour Ave Exceeded 50 pphm	106 Times:	9.2 Percent(b)
	24-Hour Ave Exceeded 10 pphm	121-124 Times:	79.6-81.6 Percent(a)
	24-Hour Ave Exceeded 14 pphm	99-101 Times:	65.1-66.5 Percent(b)
	1-Hour Peak 461-465	3-Hour Peak 294-295	24-Hour Peak 72

*pphm SO_2 monitored near plot center at canopy height
(a) violates Montana standards
(b) violates Federal standards

TABLE 6.2. ZAPS II SEASONAL SUMMARY, 1978*

Control   Geometric Mean = 0.9-1.2   Standard Geometric Deviation = 1.3,2.3   Arithemtic Mean = 1.1-1.2

1-Hour Ave Exceeded 25 pphm	0 Times:	0 Percent
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 14 pphm	0 Times:	0 Percent

1-Hour Peak 6   3-Hour Peak 4   24-Hour Peak 2.2

Low   GM = 2.5   SGD = 2.3   Arithmetic Mean = 4.0

1-Hour Ave Exceeded 25 pphm	5 Times:	.2 Percent
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	2 Times:	1.3 Percent(a)
24-Hour Ave Exceeded 14 pphm	0 Times:	0 Percent

1-Hour Peak 30   3-Hour Peak 22   24-Hour Peak 11

Medium   GM = 4.6   SGD = 2.6   Arithmetic Mean = 8.0

1-Hour Ave Exceeded 25 pphm	240-243 Times:	7.2 Percent(a)
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	44-45 Times:	28.0-28.6 Percent(a)
24-Hour Ave Exceeded 14 pphm	10-11 Times:	6.4-7.0 Percent(b)

1-Hour Peak 57   3-Hour Peak 47   24-Hour Peak 20

High   GM = 9.2-9.3   SGD = 2.8,2.9   Aritnmetic Mean = 17.8

1-Hour Ave Exceeded 25 pphm	648 Times:	19.3 Percent(a)
3-Hour Ave Exceeded 50 pphm	65-67 Times:	5.8-5.9 Percent(b)
24-Hour Ave Exceeded 10 pphm	119 Times:	75.8 Percent(a)
24-Hour Ave Exceeded 14 pphm	97-98 Times:	61.8-62.4 Percent(b)

1-Hour Peak 125   3-Hour Peak 89   24-Hour Peak 44

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*pphm SO_2 monitored near plot center at canopy height
(a) violates Montana standards
(b) violates Federal standards

TABLE 6.3. COMPARISON OF WHOLE SEASON SUMMARY STATISTICS FOR SO_2 CONCENTRATIONS DURING DAYLIGHT HOURS AND NIGHTTIME HOURS ON ZAPS*

Plot	ZAPS I		ZAPS II	
	Day	Night	Day	Night
Control				
Geometric Mean	1.0	1.4	1.2	1.3
Std. Geo. Dev.	1.4	2.0	1.2	1.3
Arithmetic Mean	1.1	1.9	1.2	1.4
1 hour peak	26.0	17.8	6.0	6.1
Low				
Geometric Mean	1.9	4.7	1.8	4.1
Std. Geo. Dev.	2.0	2.9	1.7	2.5
Arithmetic mean	3.0	8.4	2.28	6.4
1 hour peak	98.5	109.3	24.38	30.7
Medium				
Geometric Mean	3.1	9.6	3.2	8.0
Std. Geo. Dev.	2.3	3.2	2.0	2.7
Arithmetic Mean	5.9	19.2	4.5	13.1
1 hour peak	145.6	181.8	44.5	62.9
High				
Geometric Mean	6.3	14.0	6.5	15.5
Std. Geo. Dev.	2.6	3.4	2.3	3.1
Arithmetic Mean	11.4	29.3	10.2	28.5
1 hour peak	418.0	442.9	92.7	147.9

*pphm SO_2, HIGH run

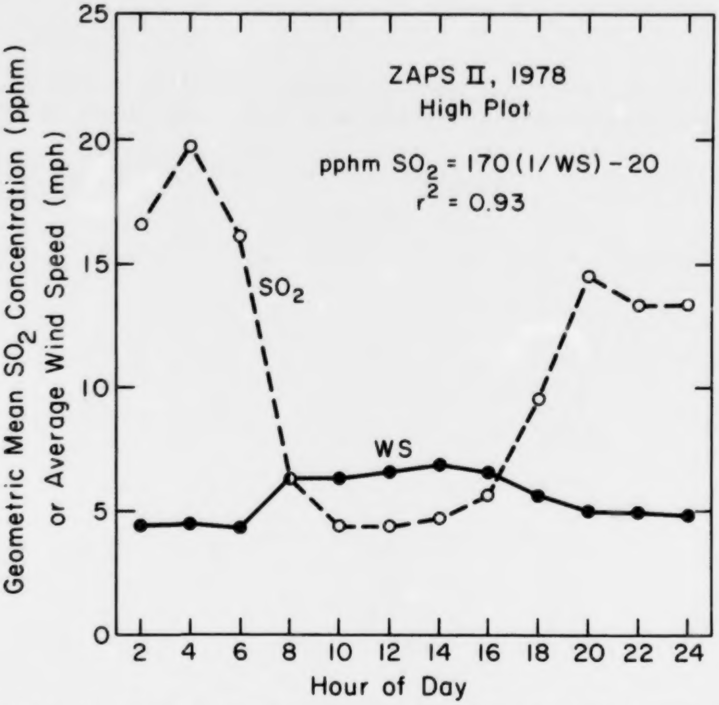

Figure 6.1. Diel Cycle of wind speed and SO_2 levels (seasonal averages)

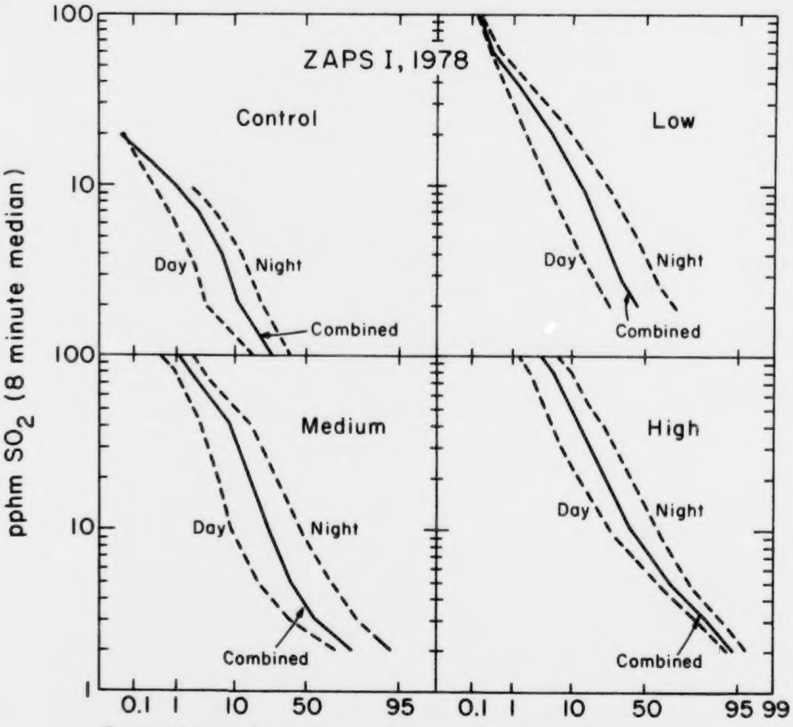

Figure 6.2. Frequency distributions of SO_2 concentrations on ZAPS I, 1978.

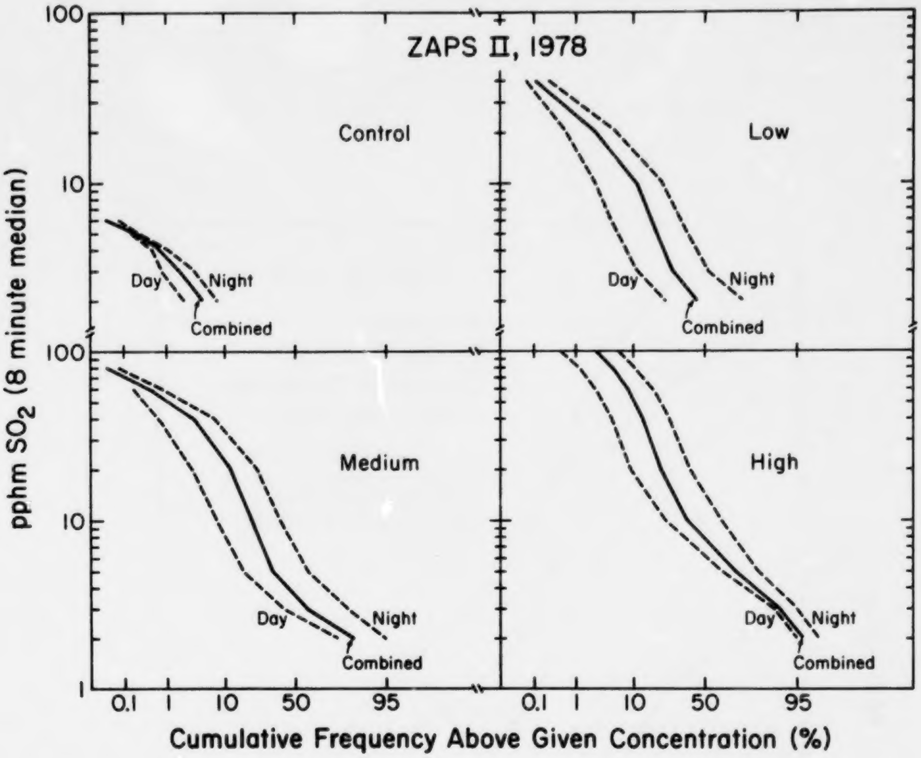

Figure 6.3. Frequency distributions of SO_2 concentrations on ZAPS II, 1978.

and diel variation have been presented. The potential significance of the substantial difference between day and night exposure regimes has not been emphasized.

Most organisms have diel activity cycles and consequently are likely to be affected more at certain times of day than at others. The biologically effective SO_2 exposure for green plants must be radically different from that for nocturnal rodents. In developing relationships betweeen exposure concentrations and effects (dose-response), differences in biologically effective exposures among various organisms must be taken into consideration. In general, day active organisms received substantially lower exposure at canopy height than did night active organisms. The biologically effective exposures of day-active organisms may be substantially less than implied by the overall seasonal summary data. (Figures 6.2 and 6.3, Table 6.3)

The Diel Pattern of SO_2 Concentrations Near "Real World" Sources

Area sources, such as urban centers, tend to have a bimodal pattern of daytime SO_2 concentrations with peaks at mid-morning and late afternoon followed by low, constant night time levels (Holzworth, 1973; Munn and Katz, 1959). The diurnal SO_2 concentration patterns are primarily determined by variations in mixing, wind speed and source strength. Concentrations in the vicinity of point source are in addition to these factors, highly dependent upon wind direction. Plume strikes are likely to be more frequent during the

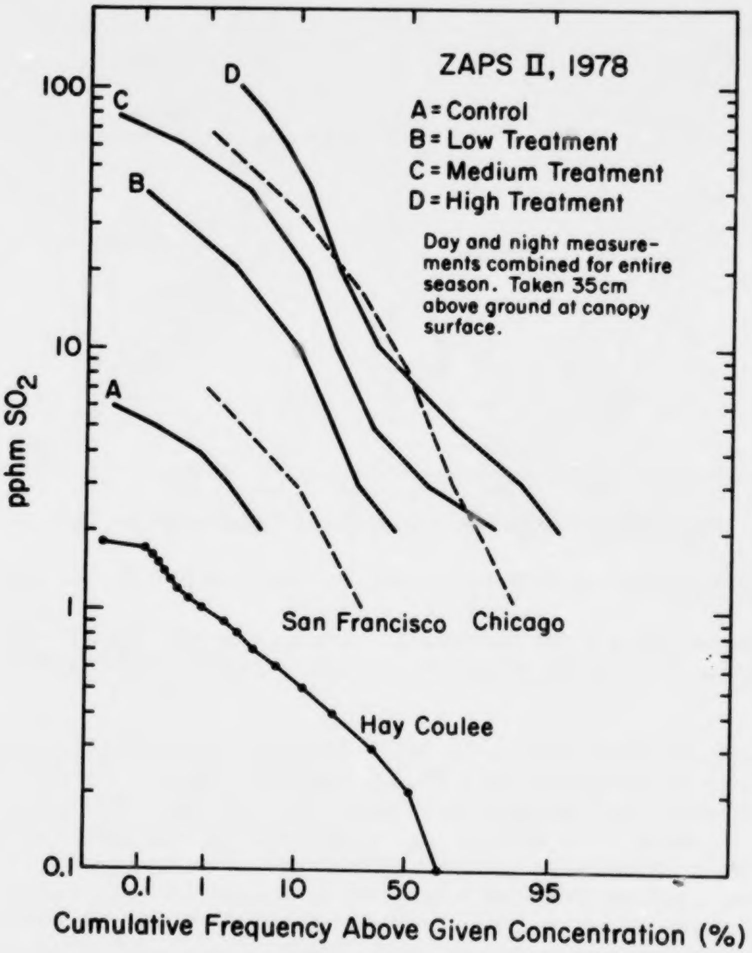

Figure 6.4. Frequency distributions of SO_2 concentrations monitored on ZAPS II, 1978 compared with those monitored in Chicago, San Francisco, (1962-67) (HEW, 1970), and Hay Coulee (7.5 Miles S.E. of Colstrip power plants, 1977-79).

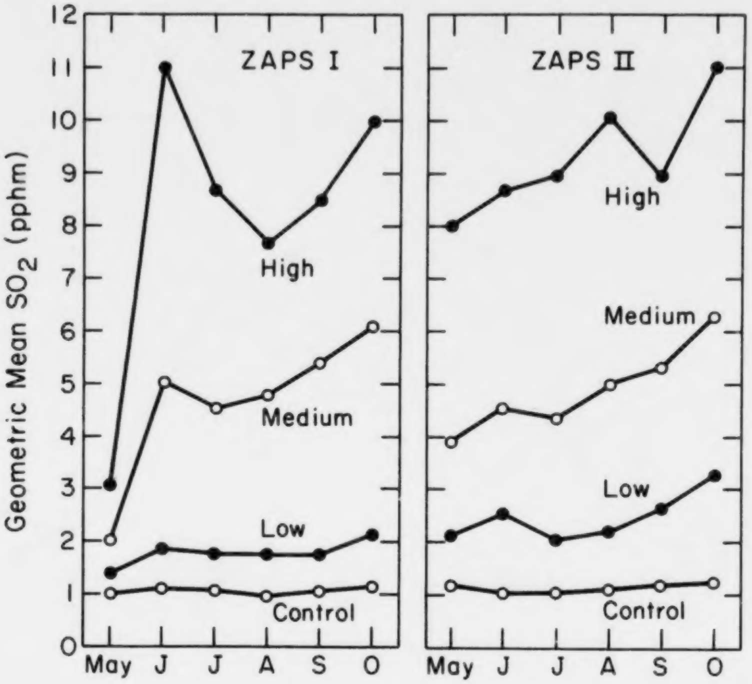

Figure 6.5. Monthly variation in geometric mean SO_2 concentrations for 1978 (HIGH run).

daytime because conditions often favor mixing while at night stable conditions tend to prevent mixing of the plume to the ground (Smith *et al.*, 1979). The most probable concentration near a point source is zero. High SO_2 levels are rare and unpredictable.

The diel pattern of the frequency of elevated SO_2 concentrations measured at Hay Coulee, 7.5 miles southeast of Colstrip, is unimodal with plume strikes occurring most frequently near midday and gradually decreasing in frequency to very low levels at night (Figure 6.6) (Ludwick *et al.*, section 1). This is the inverse of the diel pattern of concentrations observed on the ZAPS plots. At Hay Coulee, an elevated SO_2 concentration is dependent upon wind transport from the stacks at Colstrip. Plume strikes tend to occur during the day when wind speeds are elevated and turbulent mixing brings the plume to the ground. On ZAPS, on the other hand, dilution at elevated wind speeds causes SO_2 concentrations to decrease during the day. Here the canopy lies within 35 cm of the SO_2 sources and the importance of wind in plume transport is less than at Hay Coulee.

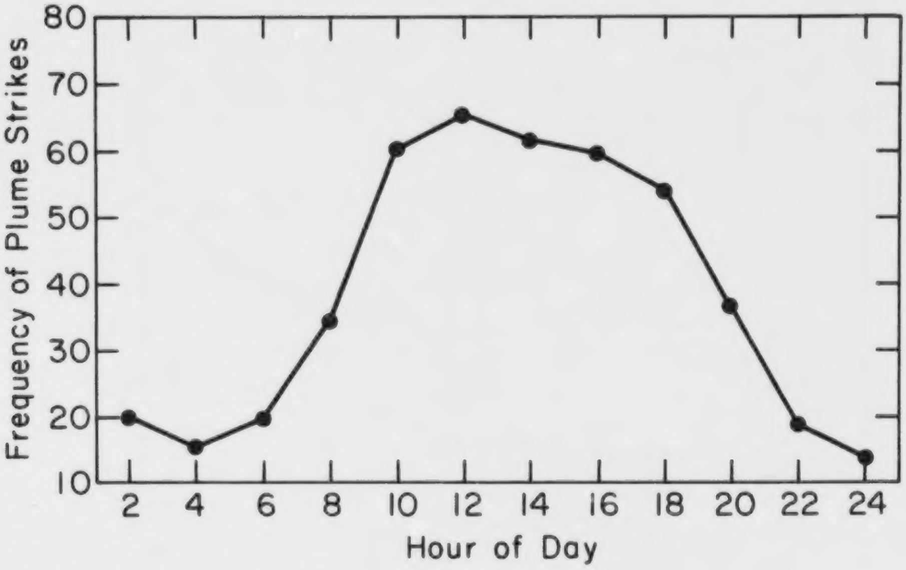

Figure 6.6. Frequency of intercepts of the Colstrip plume at Hay Coulee (7.5 miles S.E. of the stacks).

Though the ZAPS exposure regime appears to simulate the shape of the concentration frequency distribution curve at Hay Coulee (Figure 6.4) reasonably well, it does not replicate the diel cycle of this point source. The ZAPS exposure regime is expected to simulate an area source with greater fidelity. Sulfur dioxide concentrations in an area-wide plume could be expected to have the same relationship with wind speed as those observed on ZAPS.

CONCLUSIONS

At canopy height, daytime SO_2 concentrations on ZAPS are substantially lower than nighttime concentrations. The diel pattern of SO_2 concentrations does not resemble that induced by the Colstrip power plants but should resemble patterns generated by area sources. Diel activity cycles of organisms on the plots must be considered in defining dose-response relationships.

REFERENCES

Holzworth, G.C. 1973. Variations of Meteorology, Pollutant Emissions, and Air Quality. In: 2nd Joint Conf. Sensing Environ. Pollut., American Chemical Society, Washington, D.C. pp. 247-255.

Lee, J.J., R.A. Lewis and D.E. Body, 1976. The Field Experimental Component: Evaluation of the Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Second Interim Report, Colstrip, Montana, R.A. Lewis, N.R. Glass and A.S. Lefohn, eds. EPA 600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon, pp. 188-202.

Lee, J.J. and R.A. Lewis. 1978. Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Third Interim Report, Colstrip, Montana, E.M. Preston, and R.A. Lewis eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon, pp. 322-344.

Lee, J.J., E.M. Preston and R.A. Lewis. 1978. A System for the Experimental Evaluation of the Ecological Effects of Sulfur Dioxide. In: 4th Joint Conf. Sensing Environ. Pollut., American Chemical Society, Washington, D.C. pp. 49-53.

Lee, J.J., E.M. Preston, and D.B. Weber. 1979. Temporal Variation in SO_2 Concentration on ZAPS. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana, E.M. Preston and T.L. Gullett. eds. EPA 600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Oregon, pp. 284-305.

Munn, R.E. and M. Katz. 1959. Daily and Seasonal Pollution Cycles in the Detroit-Windsor Area. Internat. J. Air Poll., 2:51.

Smith, T.B., D.L. Blumenthal, J.A. Anderson, and A.H. Vanderpol. 1979. Transport of SO_2 in Power Plant Plumes: Day and Night. Atmos. Environ., 12:605-611.

SECTION 7

VERTICAL SO_2 CONCENTRATION PROFILE ON ZAPS DURING THE 1978 FIELD SEASON

L. T. K. Wong, E. M. Preston, and T. L. Gullett

ABSTRACT

During July and August of 1978, the vertical profile of SO_2 concentrations within the vegetation canopy was studied. At a central point on each treatment plot, SO_2 concentrations were measured hourly at approximate ground level, mid-canopy and canopy surface with a flame photometric SO_2 analyzer. Vertical stratification in SO_2 concentration was pronounced at night and minimal during daytime. Geometric mean SO_2 concentration at median vegetation height during daylight was approximately 1.4, 1.8, and 5.8 pphm on ZAPS I and 1.4, 1.8, and 2.8 pphm on ZAPS II Low, Medium, and High plots respectively. These concentrations are substantially lower (25%-70%) than the canopy surface exposures reported in Section 6. The SO_2 vertical profiles are reasonably similar to those which might be expected during fumigation from a coal-fired power plant plume.

INTRODUCTION

A detailed description of ZAPS can be found in Lee and Lewis (1978), and in the preceding section. Because the ZAPS is an open system covering a large land area, variations in SO_2 concentration in vertical and horizontal space and time may occur. In Section 6, horizontal distribution of SO_2 on the ZAPS plots was discussed. In this section, vertical distribution of SO_2 within the plant canopy is characterized.

During the 1977 field season, the vertical profile of concentration on the ZAPS plots was estimated using Huey sulfation plates (Preston and Gullett, 1979). The plates absorb ambient SO_2 and retain it as lead sulfate. The amount of sulfate recovered after a given exposure period allows estimation of relative exposure rates among sample locations. Results indicated that SO_2 concentrations were highest near the top of the plant canopy and decreased with increasing depth within the canopy (Preston and Gullett, 1979).

This vertical stratification of SO_2 has important implications for quantification of exposure dose since organism activity is also stratified within the canopy and this stratification may vary temporally. Therefore, during the 1978 field season, diel variation in the pattern of vertical SO_2 stratification was investigated.

MATERIALS AND METHODS

Real time sulfur dioxide concentrations at 1, 15 and 31 cm above the ground were measured at a common central point on all plots during the 1978 growing season. With minor changes (see Section 6), the procedures described in Lee *et al*. (1979) were followed. Measurement periods are shown in Table 7.1.

Air temperature, relative humidity, and wind speed and direction were also monitored with procedures described in Dodd *et al.* (1979).

TABLE 7.1. SO_2 CONCENTRATION MEASUREMENT PERIODS ON ZAPS, 1978

	Treatment Plots		
ZAPS	High Treatment	Medium Treatment	Low Treatment
I	7/26/78 → 8/29/78 (34 days)	7/13/78 → 7/26/78 (14 days)	6/27/78 → 7/13/78 (17 days)
II	7/14/78 → 7/27/78 (14 days)	7/27/78 → 8/08/78 (13 days)	8/09/78 → 8/28/78 (19 days)

RESULTS AND DISCUSSION

Diel Variation in the SO_2 Profile

Sulfur dioxide concentrations averaged over the entire monitoring period for each hour for each plot at 1, 15 and 31 cm above the ground are shown in Figures 7.1 and 7.2. In general, SO_2 concentrations were highest near the top of the plant canopy and decreased with height. However, the vertical concentration profile varied over the diel cycle. Significant vertical stratification of SO_2 only occurred at night when SO_2 concentrations at the canopy surface were high. During daytime, concentrations were lower and vertical differences were small.

The observed diel variation in vertical SO_2 concentration profile is consistent with theoretical expectations based on diel changes in micrometeorological conditions on the ZAPS plots. SO_2 concentration is expected to decrease towards the ground because the ground and vegetation are sinks for the pollutant. The magnitude of the concentration gradient should be inversely related to the degree of air turbulence near the ground. Wind speed

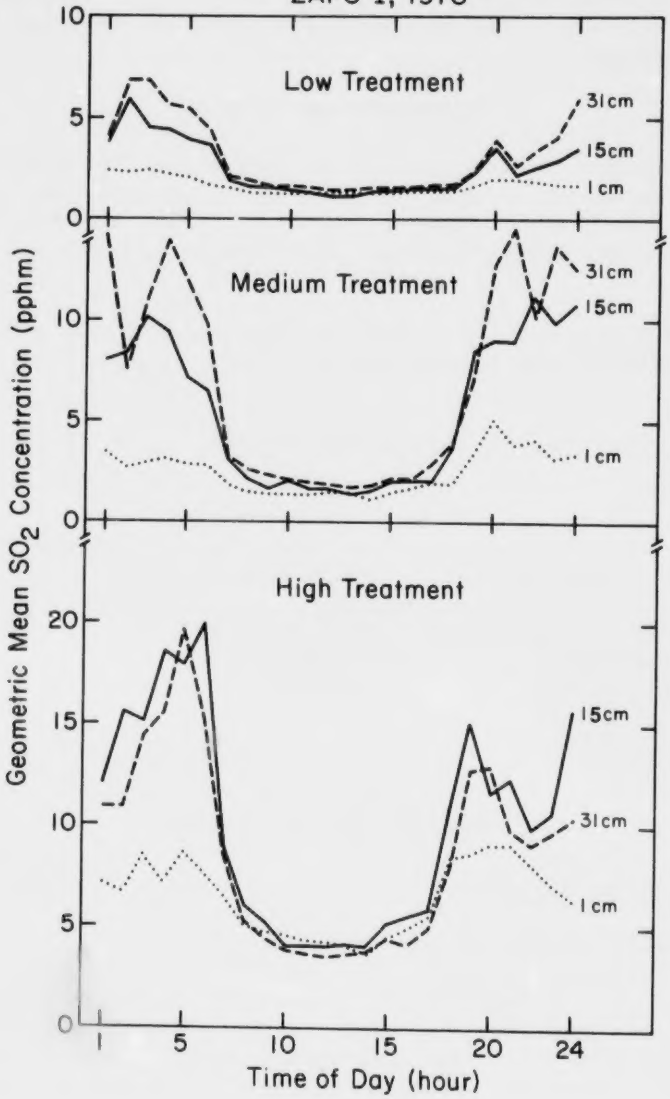

Figure 7.1. Diel variation in the SO_2 profile on ZAPS I, 1978.

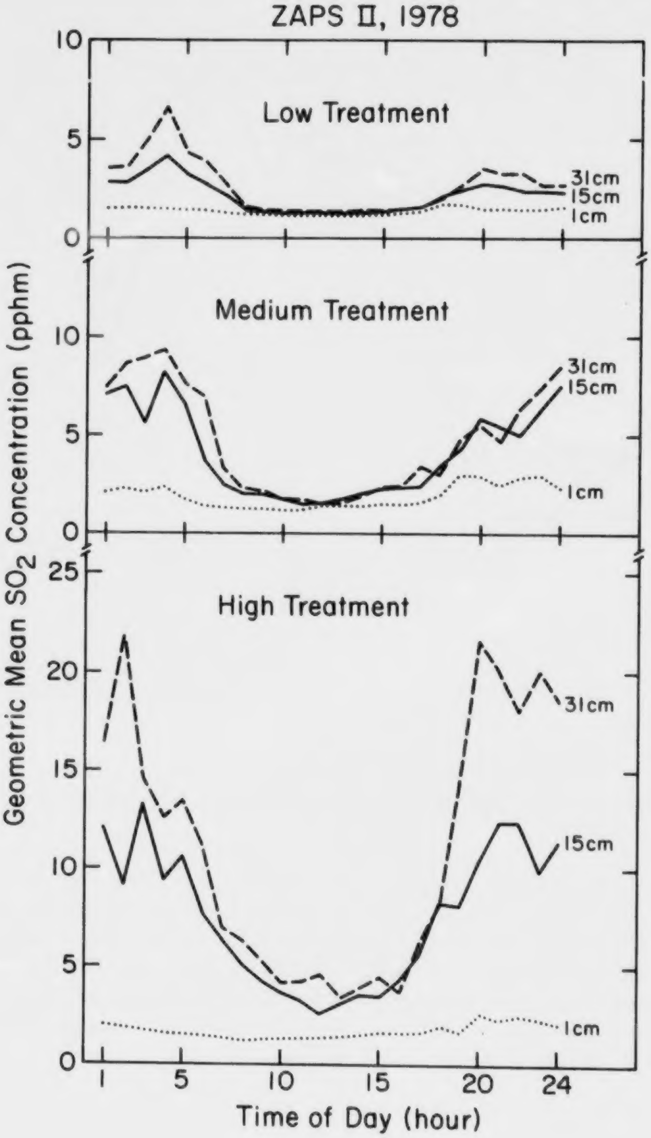

Figure 7.2. Diel variation in the SO_2 vertical profile on ZAPS II, 1979.

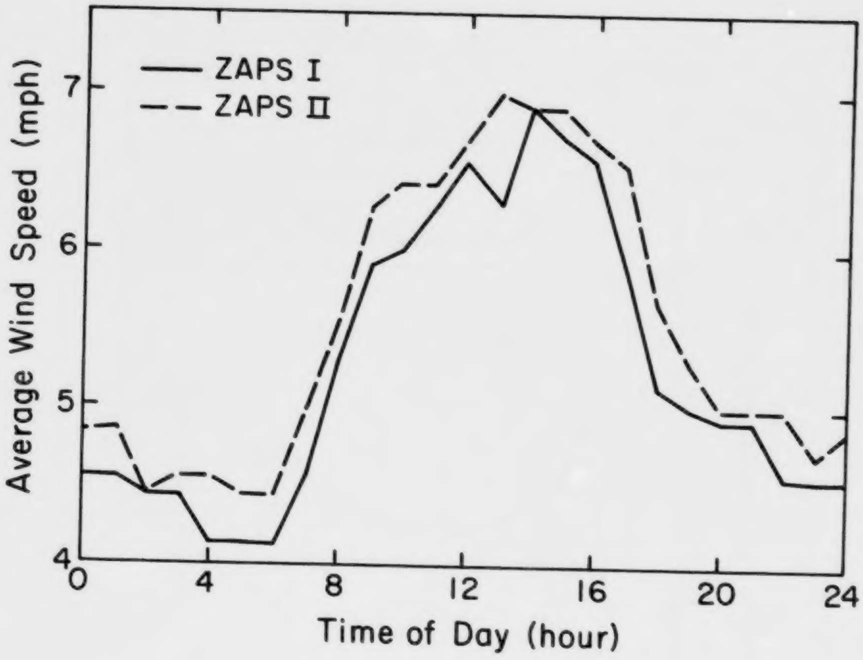

Figure 7.3. Diel variation in wind speed on ZAPS I and II, 1978.

at the ZAPS sites was higher during daytime (Figure 7.3). The greater winds during the day increased the dilution of SO_2 and resulted in lower daytime concentration.

The correlation between hourly average wind speed and hourly average SO_2 concentration on the ZAPS plots was estimated. Because the only wind speed measurement was taken 210 cm above the ground, it was necessary to extrapolate wind speed from 210 cm to 1, 15 and 31 cm above the ground. This was done using equations developed by Ripley and Redmann (1976) for a grassland similar to the ZAPS plots. The equations were generated by curve fitting wind speed measured at various heights above the ground.

Wind speed above the canopy:

$$u(Z) = u(h) \{\ln [(Z-d)/Z_o] / \ln[(h-d)/Z_o] \} \quad Z \geqq h \qquad (1)$$

Wind speed within the canopy:

$$u(Z) = u(h) \exp\{-[0.385 (Z/h) + 0.72]\, L(Z)\}\quad Z \leq h \qquad (2)$$

where u = wind speed, ms^{-1}
Z = height above ground
d = zero plane displacement, ∿ 10 cm
Z_o = aerodynamic roughness length, ∿ 2.7 cm
h = canopy height, ∿ 17.5 cm
$L(Z)$ = total leaf area index above height Z

If the wind speed at one height above the canopy is known, the vertical wind profile can be estimated from these two equations.

Results of correlation analysis for representative treatment plots IB (low treatment) and IID (high treatment) are summarized in Table 7.2. As expected, SO_2 concentration was roughly inversely proportional to the wind speed.

Condition of the vegetation surface may also influence the vertical SO_2 gradient. Wetness of the vegetation surface influences SO_2 uptake rates. Because of higher relative humidity and dew formation at night, greater SO_2 uptake rates than during daytime may occur even though stomatal resistance is less during daytime. Greater uptake rates at night should enhance the vertical concentration gradient.

TABLE 7.2. WIND SPEED AND SO_2 CONCENTRATION CORRELATION

	Correlation Coefficients					
	Wind speed (u) and SO_2 concentration (c) for height (z), u(z) c(z)					
Plot	c(31) u(210)	c(15) u(210)	c(1) u(210)	c(31) u(31)	c(15) u(15)	c(1) u(1)
ZAPS IB	-0.855	-0.862	-0.901	-0.851	-0.867	-0.883
ZAPS IID	-0.823	-0.891	-0.867			

Thus the observed diel variation in the vertical profile in SO_2 concentration can be explained from meteorological factors alone. However, the vertical concentration gradient should also be enhanced by the ZAPS exposure system. Sulfur dioxide concentration is dependent upon distance from the source and thus should decrease between the exposure pipe network and the ground unless wind speed is sufficient to keep the air mass between the network and the canopy surface well mixed. This artifact of ZAPS design would also enhance the vertical SO_2 gradient at night when winds are calm.

Effective Exposure for Grasses

Figure 7.4 shows the mean profiles of daytime (6 a.m.-6 p.m.) and night-time (7 p.m.-5 a.m.) SO_2 concentrations for the ZAPS plots. Since the uptake of SO_2 by plants is greatest during daytime when the stomata are open, their effective exposure dose is more nearly characterized by the day curves.

Effective exposure dose in grasses is also influenced by the vertical distribution of leaf area. Vertical differences in SO_2 concentration results in non-uniform exposure to leaves at different heights. Cumulative percentage of total leaf area as a function of height above the ground for a grassland similar to the ZAPS plots is given in Figure 7.5 (Ripley and Redmann, 1976). Fifty percent of total leaf area is found below 6.5 cm above the ground and 95% below 20 cm. The median effective daytime exposure for grasses on the ZAPS plots was estimated by the daytime geometric mean SO_2 concentration at 6.5 cm for each treatment plot (Table 7.3). These are substantially lower (25-70%) than the canopy surface concentrations reported in Section 6.

TABLE 7.3. EFFECTIVE DAYTIME SO_2 EXPOSURE (PPHM)

	Low Treatment	Medium Treatment	High Treatment
ZAPS I	1.40 (0.48)*	1.85 (0.64)	5.75 (0.25)
ZAPS II	1.45 (0.39)	1.75 (0.56)	2.80 (0.70)

* Proportional decrease from combined day-night canopy surface concentrations computed as reported in Section 6.

Comparison with other Area and Point Sources

It is of interest to know if the pattern of vertical stratification in SO_2 concentrations within the vegetation canopy observed on ZAPS is reasonably similar to that which might be expected in "real world" pollution fumigations.

Unfortunately, appropriate field data for comparison are lacking. The major interest has been the estimation of SO_2 flux to the canopy surface (Shepherd, 1974; Owers and Powell, 1974). Extrapolation of above canopy SO_2 concentration profile into the canopy is not possible because the turbulent structure of the air flow changes inside the canopy (Inoue, 1963). Moreover, the effect of SO_2 uptake by plants can be significant (Bennett and Hill, 1973).

In the absence of field data, vertical SO_2 concentration profile within a grass canopy was estimated theoretically. Based on the assumption that SO_2 concentration within canopy depends on wind speed and leaf surface abundance, a semi-black box model was constructed.

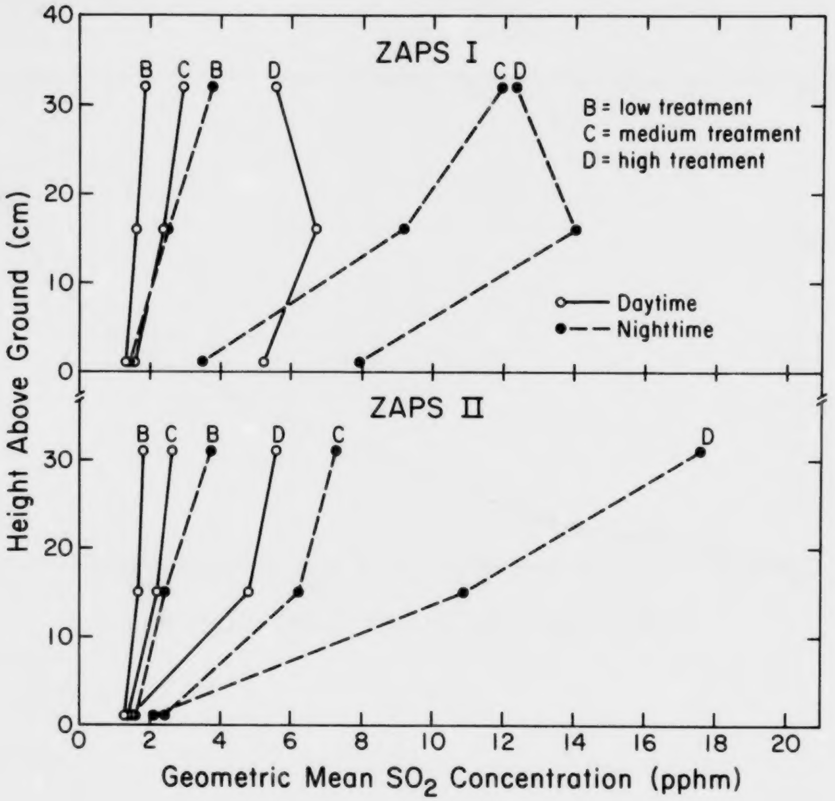

Figure 7.4. Mean SO_2 vertical profiles for daytime (6 a.m. - 6 p.m.) and nighttime during the study periods.

$$C(Z) = C(h) \; [1 - \sqrt{\frac{LAI(h) - LAI(Z)}{LAI(h)} \cdot \frac{WS(h) - WS(Z)}{WS(h)}} \qquad (3)$$

where C = SO_2 concentration (pphm)
Z = height above ground (cm)
h = height of canopy (cm)
LAI = leaf area index (Ripley and Redmann, 1976)
WS = wind speed

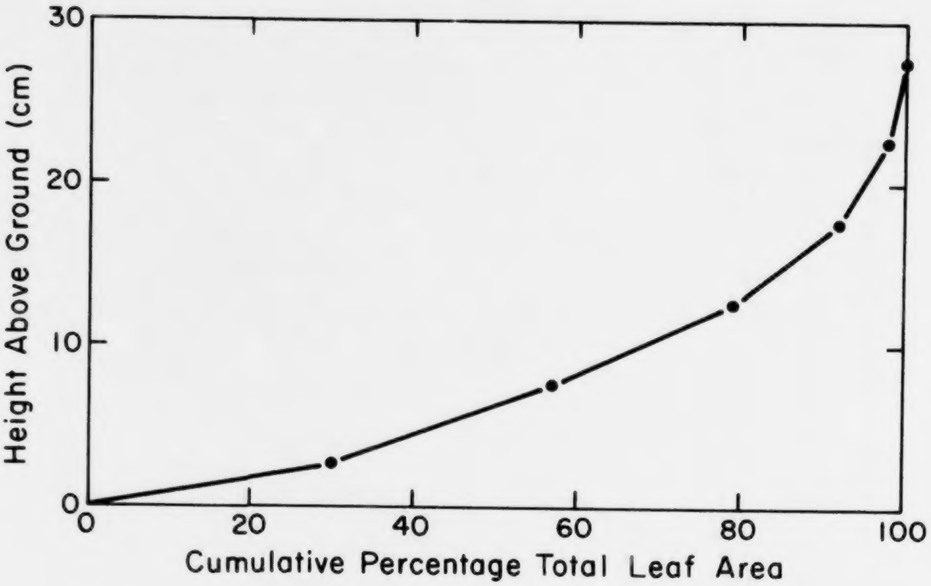

Figure 7.5. Vertical distribution of leaf area exposed to SO_2 at various heights above ground from Ripley and Redmann (1976).

Given the SO_2 concentration at canopy height, the model predicts the vertical SO_2 concentration profile within the canopy. Predictions from this simple semi-black box model agree reasonably well with those from a more complex mechanistic model developed at Colorado State University (Heasley, personal communication, 1980), and with data from chamber measurements (Bennett and Hill, 1973). The model was used to estimate the SO_2 concentration profiles near a coal-fired power plant. The following above canopy SO_2 concentrations were assumed to be typical for concentrations near a large coal-fired power plant.

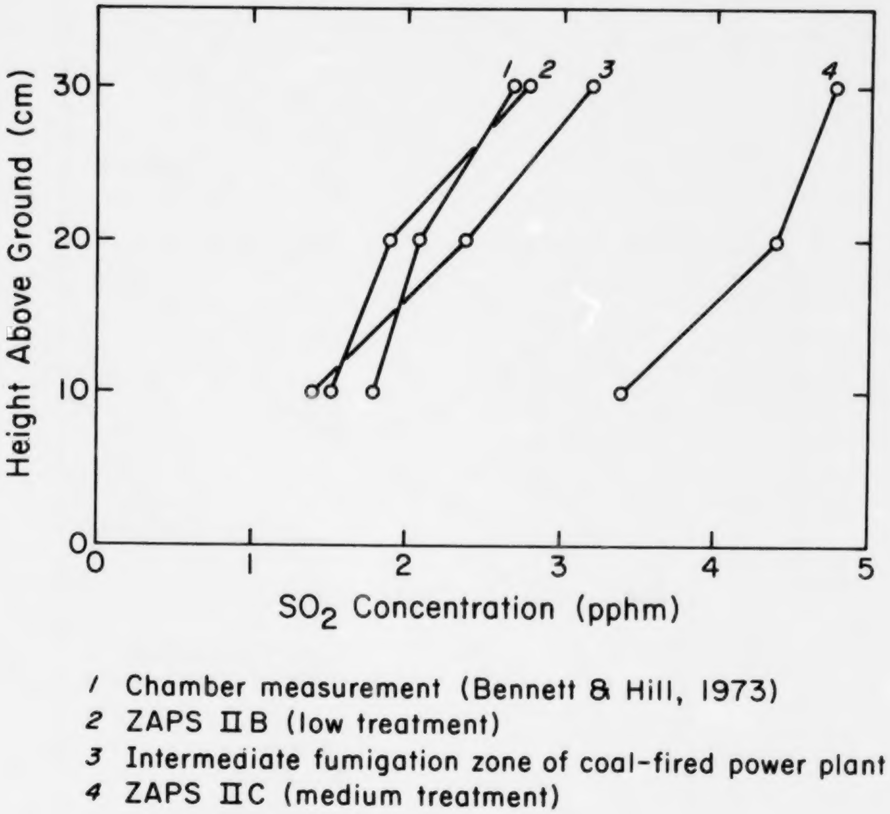

Figure 7.6. Comparison of the vertical SO_2 concentration profiles on grassland canopies measured on selected ZAPS treaments during chamber study, and expected in the vicinity of a coal-fired power plant.

Fumigation Zone	Annual Average SO_2 Concentration (pphm)
Inner	5.0
Intermediate	3.0
Outer	1.0

Wind speed profile was estimated as described earlier. With these data, the vertical SO_2 concentration profile for the inner, intermediate and outer fumigation zones of a coal-fired power plant was computed from Equation 3. The result is compared with the vertical SO_2 concentration profiles observed on the ZAPS plots in Figures 7.6 and 7.7. There is a general similarity

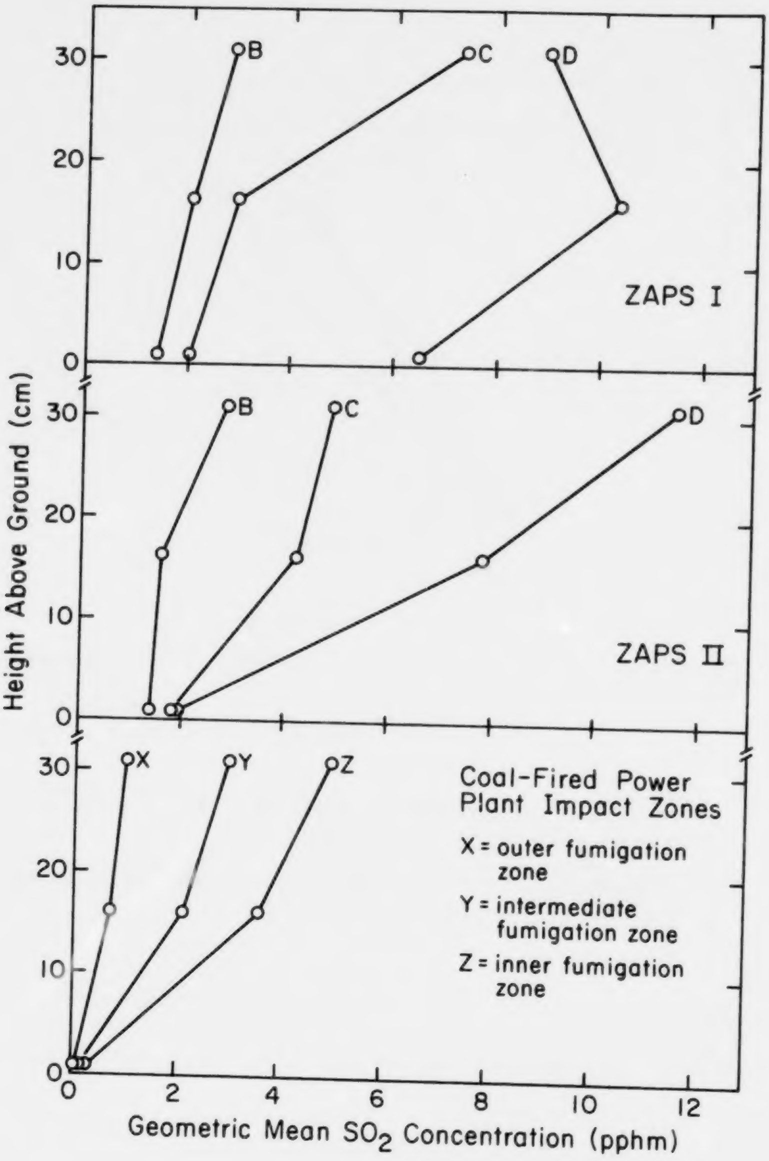

Figure 7.7. Comparison of vertical SO_2 concentration profiles observed on ZAPS with estimates of those expected in the vicinity of a coal-fired power plant.

between the shape of the vertical SO_2 concentration curves on ZAPS, chamber experiment, and coal-fired power plant fumigation zones. The low treatment and medium treatment plot on ZAPS are comparable respectively to the outer and intermediate fumigation zone. The high treatment plot has a higher SO_2 concentration than the inner fumigation zone.

CONCLUSIONS

Vertical stratification of SO_2 concentrations within the grassland canopy on treatment plots is pronounced at night but small during daytime when wind speed is elevated. The vertical profiles of SO_2 concentration are reasonably similar to those which might be expected during fumigation from a coal-fired power plant plume.

REFERENCES

Bennett, J. H. and A. C. Hill. 1973. Absorption of Gaseous Air Pollutants by a Standardized Plant Canopy. J. Air Pollut. Control Assoc., 23:203-206.

Dodd, J. L., W. K. Lauenroth, R. G. Woodmansee, G. L. Thor, and J. Chilgren. 1979. Soil, Chemical, and Meteorological Characteristics at ZAPS. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 332-390.

Inoue, E. 1963. On the Turbulent Structure of Airflow within Crop Canopies. J. Meteorol. Soc. Jap. 41(6):317-325.

Lee, J. J. and R. A. Lewis. 1978. Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana. E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 322-344.

Lee, J. J., E. M. Preston, and D. B. Weber. 1979. Temporal Variation in SO_2 Concentration on ZAPS. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 284-305.

Owers, M. J. and A. W. Powell. 1974. Deposition Velocity of Sulphur Dioxide on Land and Water Surfaces Using a ^{35}S Tracer Method. Atmos. Environ., 8:63-67.

Preston, E. M. and T. Gullett. 1979. Spatial Variation of Sulfur Dioxide Concentration on ZAPS During the 1977 Field Season. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 306-331.

Ripley, E. A. and R. E. Redmann. 1976. Grassland. In: Vegetation and the Atmosphere, Vol. 2. Case Studies, J. L. Monteith, ed. Academic Press, London. pp. 349-398.

Shepherd, J. G. 1974. Measurements of the Direct Deposition of Sulphur Dioxide onto Grass and Water by the Profile Method. Atmos. Environ., 8:69-74.

SECTION 8

MYCORRHIZAL ASSOCIATION AND ROOT CHARACTERISTICS IN WESTERN WHEATGRASS FUMIGATED WITH SULFUR DIOXIDE

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ABSTRACT

The degree of *Agropyron smithii* (western wheatgrass) root tissue infection by beneficial symbionic fungi belonging to the group (Endogonaceae) of vesicular-arbuscular endomycorrhizae declines as leaf sulfur content increases with sulfur dioxide fumigation. The proportion of vesicles increases while arbuscules, the nutrient transfer stage of the endophyte, decline as a function of leaf sulfur load, suggesting the normal balance between the symbionts may be altered. Increased root senescence results with increases in leaf sulfur and root sulfur. *A. smithii* culms are larger, roots more vigorous and infection is higher on two control plots (OPC 1 and OPC 2) than a third control (ZAPS IIA) possibly as a consequence of the latter still recovering from grazing pressure and receiving some sulfur dioxide fumigation. The quantification of infection levels by absorbance determination cannot be applied with consistent reliability to long term field studies.

INTRODUCTION

Endomycorrhizae are a fungal group which form a beneficial symbiosis with the root systems of many higher plants, including grasses, forest trees and many food crops (Baylis, 1974). The host plant provides carbohydrates to sustain the fungus. The primary advantage to the host is an increased ability to extract mineral nutrients from the soil, especially phosphorous (Sanders and Tinker, 1973). Increased nodulation and nitrogen fixation (Richards and Voigt, 1964), disease deterrence (Zak, 1964) and more efficient soil water extraction (Safir *et al.*, 1971) are among the numerous additional benefits. Ruehle and Marx (1979) have summarized the importance of fungal root symbionts to plants of economic importance.

The hyphae of endomycorrhizae actually penetrate the feeder roots and enter the cortex of the host plant roots. This is a controlled entry and does not normally disrupt the morphological structure of the host. In addition to the hypae, two distinct structures, vesicles and arbuscules, are formed within the host cells. Vesicles, which are found externally as well as within the roots, are a spherical enlargement of a hyphal strand. The vesicles are considered to serve as both food storage organs and infectious spores (Kinden and Brown, 1975). Arbuscules are formed by a multitudinous branching of the hyphae in a localized area and are believed to be the mechanism which transfers mineral nutrient from the fungus to the host (Cox and Tinker, 1976). The vesicles, arbuscules and internal growth habit of these root-associated fungi are the basis for referring to this group as vesicular-arbuscular (VA) endomycorrhizae (EDM). We have reviewed the biology of these organisms as it relates to this study in an earlier report (Rice *et al.*, 1979).

There has been little study of the responses of mycorrhizae to air pollutants. Ectomycorrhizae provide an analogous function in terms of mineral nutrition for their host, but do not actually penetrate the roots as do the endomycorrhizae. Grzywacz (1964) is cited in McCool *et al.*, (1979) as reporting a decline in the frequency of fungal association with the roots of forest trees subjected to sulfur dioxide and fluoride pollution. An investigation of the ectomycorrhizae on spruce (Sobotka, 1964 in Heagle, 1973) stressed by sulfur dioxide also reports a detrimental impact on the symbiosis. Recent work at the Statewide Air Pollution Research Center, Riverside, California, has demonstrated that the endomycorrhiza *Glomus fasciculatus* associated with tomato plants is sensitive to both sulfur dioxide and nitrogen dioxide (Granett, 1978). Also working at Riverside, McCool *et al.*, (1979) reported reductions in infection and spore production of *G. fasciculatus* associated with citrange when the host plant was fumigated with ozone.

In 1977, a study was conducted at the Zonal Air Pollution Systems (ZAPS) site to determine whether endomycorrhizae were associated with prairie grasses occupying the sites and whether the association was influenced by sulfur dioxide treatment. The results of the investigation are reported by Rice *et al.*, (1979) and are briefly summarized here:

(1) Vesicular-arbuscular endomycorrhizae are present with several prairie grass species including *Agropyron smithii* (western wheatgrass).

(2) Infection levels declined with increasing ambient sulfur dioxide treatments and leaf sulfur concentrations.

(3) Swollen interior vesicles and excessive numbers of exterior vesicles were observed on the low treatment plots, suggesting a disruption of the normal balance between the symbionts.

(4) A colorimetric method for determining infection levels (Becker and Gerdemann, 1977) was correlated with microscopically determined levels of infection and thus, potentially could be used as a much less labor-intensive assay technique.

This study was continued through 1978 to further refine the methods and confirm the 1977 observations. The collection and preparation of *A. smithii* root samples for mycorrhizae assays also provided an opportunity to investigate a number of other root and shoot characteristics.

MATERIALS AND METHODS

The ZAPS II site consists of four .5 hectare plots fumigated with sulfur dioxide for the third growing season in 1978. Gas is delivered at different but fixed rates to three of the plots by a perforated pipe network supported 75 cm above the ground. Meteorological factors induce significant temporal variation in ambient concentrations of each of the plots, and the fourth plot receives sulfur dioxide as a consequence of wind drift from the three plots which are directly fumigated. Two additional grassland plots (Off Plot Controls, OPC), well removed from the ZAPS II site, were also sampled. This was done primarily to provide control plots which did not receive sulfur dioxide as a consequence of wind drift. The ambient sulfur dioxide concentrations of the four plots at the ZAPS II site were continuously measured at the top of the plant canopy (30 cm) by a flame photometric analyzer (See section 6, Table 6.2). In addition to temporal variation in sulfur dioxide concentrations, significant horizontal and vertical concentration gradients exist. Daytime concentrations are less than the stated mean, and the average concentration declines with descent into the plant canopy. Detailed description of the fumigation patterns are provided by Preston *et al.*, (Section 6) and Preston and Gullett (1979). Rice *et al.*, (1979) have demonstrated that sulfur accumulation by plant tissues reflects these gradients within the individual treatment plots. Therefore, stated concentrations reflect upper limits of dosage delivered.

Twenty subplots on each treatment plot were randomly chosen for sampling in June, July and September of 1978. One soil core (15 cm diameter by 20 cm deep) with intact roots and shoots was taken from each subplot. The cores were bagged in plastic and shipped to the Environmental Studies Laboratory in Missoula, Montana, for processing. Cores were refrigerated at the laboratory and processed by blocks in a random manner within one week.

The cores were trimmed to a uniform size (10 cm diameter x 15 cm deep), softened in water for one hour, and the entire *A. smithii* plants separated from the soil with a hose. The number of live culms of *A. smithii* per core and the weight of tencurrent year culms per core were recorded. Fresh root weight was determined after the recovered root mass was placed in a humidifier to obtain full turgor and then blotted dry. Sub-samples (.5 g) of the root mass were autoclaved to obtain a hot water extract for colorimetric determination (400 NM), to be used as an index of mycorrhizal infection, with an adaptation (Rice *et al.*, 1979) of a method proposed by Becker and Gerdemann (1977). An additional modification of previous techniques was the filtration of the extracts to remove any debris.

After the absorbance readings were obtained, the same root mass was cleared in KOH and treated with trypan blue in lactophenol (Phillips and Hayman, 1970) to stain the fungal elements in and on the root tissues. Infection levels were then estimated by a modification (Rice *et al.*, 1979) of Newman's (1966) point intersection method as done by Sparling and Tinker (1975). The method consists of spreading the stained root sample in a petri dish, randomly selecting ten fields of visions under 21X binocular magnification, and scoring the number of mycorrhizal or non-mycorrhizal roots intersected by a rotated ocular graticule. Mycorrhizal presence was further stratified as to arbuscules, vesicles and other signs of infection (distinctive hypae, entry points, etc.). Both the absorbance readings and visual determinations were conducted as blind tests.

The point intersection method was also used to determine total relative root length (# of intersections per .5 g sample), to assign all roots to one of three size classes (less than .5 mm, .5 mm to 1.5 mm and greater than 1.5 mm), and to establish the ratio of browning and senescent roots.

The sulfur content of live shoots and root tissues were determined for each sample item by the Leco induction method (Gordon, 1976). The variables and collections for which they were determined are summarized in Table 8.1.

The basic experimental design employed for the biological response variable was the randomized complete block form of the two-way anova. Samples were collected, processed and recorded under the format of this model. The limited size of some individual sample items and the labor-intensive nature of the scoring restricted the generation of replicate data. When replication was available, these points were pooled, and consequently interaction was assumed to be non-significant. Various transformations were employed where suitable. Zero value residuals were calculated for missing sample items, adjusted sum of squares computed (Sokal and Rohlf, 1969) and degrees of freedom reduced appropriately. The ZAPS IIA plot was considered to be the "control," and all other plots including the OPC's were contrasted pair-wise to ZIIA (Dunnett, 1955).

TABLE 8.1. SUMMARY OF THE VARIABLES QUANTIFIED IN THE ZAPS MYCORRHIZAL STUDY

VARIABLE	UNIT	COLLECTION
Sulfur in live tops	PPM	July
Sulfur in roots	PPM	July
Absorbance (400 NM)	%	June, July
Visual Infection	%	July
Vesicles	%	July
Arbuscules	%	July
Total relative root length	# of intersections	July
Small roots (<.5 mm dia.)	%	July
Medium roots (.5 mm to 1.5 mm dia.)	%	July
Large roots (> 1.5 mm dia.)	%	July
Browning and senescent roots	%	July
Culms per core	#	July
Dry Weight Per Culm	mg	September
Fresh root weight per culm	mg	July

These treatments of the data constituted a preliminary analysis. It had been anticipated that tighter control of experimental procedures based on experience gained by the 1977 work would allow meeting the assumptions of analysis of variance. Homoscedasticity was obtained for most data sets, but uncertainies about normality remain. Accordingly, non-parametric methods (Spearmann's Rank Correlation and Mann-Whitney (Wilcoxon) Rank Sum Test) were applied to the 1978 data where appropriate, as was done with the 1977 data.

RESULTS

Colorimetric Absorbance Determination of Mycorrhizal Infection

The previous study of the relationship between endomycorrhizal infection levels as estimated by microscopic inspection of roots and infection level determined by root extract absorbance at 400 NM had suggested that although absorbance was not a precise predictor of infection levels, it did have potential as an index of relative levels of infection. A significant positive correlation between infection and absorbance was not re-established for the 1978 field work ($p > .95$). The procedure had been altered to include micropore filtration of the root extract to remove plant debris. The sample processing time was also increased as a consequence of larger cores and more numerous samples. This may have affected the absorbent or increased potential light degradation of the absorbance capacity of the molecule. The absorbent has not been isolated or chemically identified, so its role in the host-fungus relationship is unknown. Also, the percentage of browning and senescent roots of *A. smithii* increased strongly with the increase in sulfur dioxide treatment. Becker and Gerdemann (1977) had suggested "This method is not recommended for long term experiments where phenolic compounds of dying roots could interfere with the absorbance of the water extract." With present methods and without identification of the chemical nature and biological role of the absorbent, the colorimetric assay appears unreliable for determining mycorrhizal infection levels in stressed field populations.

Sulfur Accumulation in Tissues

Sulfur content in live leaves of *A. smithii* from the July collection was significantly ($p \leq .01$) elevated on each of the ZAPS II treatment plots relative to the ZAPS IIA "control" plot (Table 8.2). Although OPC 1 and ZAPS IIA had similar sulfur levels in live tissue, the OPC 2 plot had a higher sulfur content ($p \leq .05$) than the site II "control." Chemical analysis of the roots indicated a trend of increasing sulfur content with increasing ambient sulfur dioxide levels, but the difference relative to the ZAPS II "control" was significant ($p \leq .01$) only for the ZAPS IID plot (Table 8.2). However, the rank correlation between sulfur in roots and live leaves indicated a very highly significant positive relationship (Spearman Rho = .4073, n = 88, $p \leq .001$). These patterns of sulfur accumulation are consistant with those observed at ZAPS in previous years.

TABLE 8.2. SULFUR CONTENT (PPM†) OF *Agropyron smithii* FOR THE JULY 1978 COLLECTION

Treatment Plot	OPC 1	OPC 2	ZAPS IIA	ZAPS IIB	ZAPS IIC	ZAPS IID
Live Leaves	1008	1106*	931	1166**	1156**	2498**
Roots	640	595	641	703	719	843*

*$p \leq .05$, Dunnett's procedure, each treatment plot is compared with the ZAPS IIA plot.

**$p \leq .01$, Dunnett's procedure, each treatment plot is compared with the ZAPS IIA plot.

†Antilog of $\overline{X}_{\log_{10}}$.

Mean plot differences separate well by treatment, but substantial differences exist in dosage accumulated within individual treatment plots (Rice *et al.*, 1979). Individual variates have substantial overlap with those of other plots. These plant-to-plant differences in sulfur accumulation reflect within-plot heterogeneity in edaphic factors, micrometeorlogical features, gas delivery system patterns and inherent plant factors. Tissue sulfur accumulation results from a physiological integration of those factors, and sulfur content should provide a more precise estimate of low level chronic sulfur dioxide loading of individual plants than stratification of biological response variables by treatment plot. The comparison by plot stratification is summarized in Table 8.3. Table 8.4 presents a rank correlation (Spearman's) matrix which frees the analysis from the constraint of plot assignment and pairs the biological responses variables with the specific sulfur tissue content of the *A. smithii* from the individual cores. The ranking also causes total linearization of the dose-response curve, assuming it does not contain multiple inflection points.

Treatment Plots Compared to ZAPS IIA

Stratification by plot reduces the number of items per sample to 20 or less. This limited sample size weakens the test for normality, making it difficult to detect deviations from the normal distribution. The non-parametric Mann-Whitney (Wilcoxon) test was used to contrast the treatment plot to the ZAPS IIA plot (Table 8.3). The best estimate of the sample standard error as derived from the preliminary two-way anova under appropriate transformations is also presented in Table 8.3. The pattern of significant differences based on this standard error and using Dunnett's procedure was quite similar, but the Mann-Whitney test is preferred because of the aforementioned uncertainies, including occasional heteroscedasticity.

TABLE 8.3. OPC AND SITE II PLOTS COMPARED TO THE ZAPS II A PLOT

Response Variable	OPC 1	OPC 2	ZAPS IIA	ZAPS IIB	ZAPS IIC	ZAPS IID	S.E.[†]
Visual Infection[‡]	55.7 (68.3)	57.8** (71.6)	47.3 (54.0)	53.4 (64.5)	47.6 (58.0)	46.6 (52.9)	±3.55
Vesicles[‡]	18.7 (9.9)	25.2 (18.2)	20.8 (12.6)	25.9 (19.1)	27.8 (21.8)	25.2 (18.2)	±3.32
Arbuscules[‡]	25.3* (18.3)	30.9*** (26.4)	17.6 (9.2)	11.8 (4.2)	14.1 (5.9)	13.9 (5.7)	±3.02
Total Relative Root Length	44.2	54.9	44.4	36.0*	44.6	43.0	±4.55
Small Roots[‡]	42.2* (45.5)	49.6*** (57.9)	34.3 31.8	28.0 22.0	34.7 32.3	35.8 34.1	±3.12
Medium Roots[‡]	35.8*** (34.3)	35.4*** (32.0)	47.1 (53.6)	47.2 (53.9)	46.5 (52.6)	46.0 (51.9)	±2.52
Large Roots[‡]	23.9 (16.4)	16.5 (8.1)	20.5 (12.2)	26.7 (20.2)	20.3 (12.0)	19.8 (11.4)	±2.89
Browning and Senescent Roots[‡]	14.3* (6.1)	9.7*** (2.8)	23.3 (15.5)	25.5 (20.0)	31.2** (26.9)	34.1*** (31.4)	±2.94
Culms/Core[§]	2.73*** (7.46)	2.96*** (8.77)	4.52 (20.48)	4.50 (20.26)	3.87 (14.99)	4.46 (19.91)	±0.303
Fresh Root Weight/Culm[§§]	1.94 (87)	1.99 (98)	1.89 (78)	1.85 (71)	1.80 (63)	1.91 (81)	±0.055
Culm Dry Weight[§§]	2.275** (188)	2.306*** (202)	2.144 (139)	2.218* (165)	2.129 (135)	2.252** (179)	±0.0377

*Significant, $p \leq .05$, Mann-Whitney (Wilcoxon) test, individual plots contrasted to the ZAPS IIA plot.

**Highly significant, $p \leq .01$, Mann-Whitney (Wilcoxon) test, individual plots contrasted to the ZAPS IIA plot.

***Highly significant, $p \leq .001$, Mann-Whitney (Wilcoxon) test, individual plots contrasted to the ZAPS IIA plot.

[†] Best estimate of sample standard error, derived from two-way anova; sample size is 20 except OPC 1 is 19.

[‡] Angular transformation, back transformation in parenthesis.

[§] Square root transformation, back transformation in parenthesis.

[§§] Log$_{10}$ transformation, original units were milligrams, back transformation in parenthesis.

TABLE 8.4. RANK CORRELATION MATRIX

	Culm Sulfur^{111}	Root Sulfur	Infection	Vesicles	Arbuscules	TRRL†	Browning	Small Roots	Medium Roots	Large Roots	Root Weight/ Culm
Root Sulfur ^{88}	.4073***	-	-	-	-	-	-	-	-	-	-
Infection	-.1786*	ns	-	-	-	-	-	-	-	-	-
Vesicles	.1990*	ns	.4051***	-	-	-	-	-	-	-	-
Arbuscules	-.2702**	ns	.4883***	ns	-	-	-	-	-	-	-
TRRL†	ns	ns	-.4225***	-.2183*	.1834*	-	-	-	-	-	-
Browning	.4705***	.4300***	-.5166***	ns	-.4794***	ns	-	-	-	-	-
Small Roots	ns	ns	ns	ns	.6005***	.6312***	-.2461**	-	-	-	-
Medium Roots	.2082*	ns	-.2079**	ns	-.5448***	-.3555***	.2880**	-.7926***	-	-	-
Large Roots	ns	ns	.2615**	ns	ns	-.5442***	ns	-.5002***	ns	-	-
Root Weight/Culm	ns	ns	.2867**	ns	.4286***	ns	-.2479***	.2703**	-.3113***	ns	-
Culms/Core	ns	.3895***	ns	ns	ns	ns	.3108***	.2051*	.1998*	ns	ns
Vesicles/ Infection	.3118***	ns									
Arbuscules/ Infection	-.2703**	ns									
Vesicles/ Arbuscules	.3201***	ns									

*p ≤ .05, Spearman's Rank Correlation.

**p ≤ .01, Spearman's Rank Correlation.

***p ≤ .001, Spearman's Rank Correlation.

Cases where a one-tailed alternative was clearly suggested by previous work are underlined.

Sample size is 119 except were superscript to the variable indicates otherwise.

†TRRL = Total relative root length.

The OPC plots are separated from each other by about 35 kilometers; however, they tend to differ similarly from the ZAPS IIA plot. Mycorrhizal infection levels are higher (the exact probability for OPC 1 versus ZAPS IIA was .08), arbuscules are more numerous and the small root fraction is larger while the medium size root fraction is reduced. Browning and senescent roots are less numerous, and the average dry weight of culms is greater. There are few significant differences detected within site II between the intentionally fumigated plots and ZAPS IIA. Total relative root length appears to be less on ZAPS IIB, culm dry weight is elevated on ZAPS IIB and ZAPS IID, and the percentage of browning and senescent roots is greater on both ZAPS IIC and ZAPS IID than the ZAPS IIA plot (Table 8.3).

Culm weights can be used only for a plot-to-plot index of relative culm size and should not be used to estimate yield or shoot/root ratios. The dry weights appear to be biased upward. The fall 1978 standing crop yield for site II plots, based on culm density x culm dry weight, ranges from 257 to 453 g/m^2, while Dodd *et al.*, (1979) measured peak current year production ranging from 80 to 120 g/m^2 for 1975 through 1977. Unless yield was unusually high for 1978, it appears that the subsampling of the September cores for culm weight determination was biased towards the larger culms.

Comparisons of ZAPS IIA with all other plots demonstrate that infection level, including arbuscular frequency, are greater for the OPC's and that although culm density is lower, plant growth is more rank and vigorous. The increase in root necrosis is the only response that clearly parallels the average sulfur dioxide treatment applied to the plots.

Biological Responses Compared to Actual Tissue Sulfur Load

The simple rank correlation matrix is arrayed in Table 8.4. Response variables are paired with the tissue sulfur content of the same plant material from which the response variable was estimated. Three mycorrhizal component ratios, vesicles/infection, arbuscules/infection and vesicles/arbuscule, are also compared with sulfur in tissues. The examination of these ratios permits the detection of changes in the status of the proportion of vesicles and arbuscules comprising the total infection.

Infection declines with increasing sulfur content of live culms ($p < .05$). This relationship remains when only the intentionally fumigated plots (ZAPS IIB, C and D) are considered ($r_s = .2572$, $n = 57$, $p < .05$). The frequency of arbuscules in the sample and the proportion of the total infection due to arbuscular presence (arbuscules/infection) decline with increasing leaf sulfur content ($p < .01$). Both the frequency of vesicles and the proportion of the total infection due to vesicles increase with leaf sulfur ($p < .05$ and $p < .001$ respectively). The net result is an overall decline in infection ($p < .05$) and an increase ($p < .001$) in the ratio of vesicles to arbuscules (vesicles/arbuscle) as a function of leaf sulfur. Significant correlation between these fungal endophyte variables and root sulfur were not observed ($p > .95$).

The results support the decline in infection with increasing leaf sulfur concentration observed in the 1977 study. Excessive numbers of exterior vesicles were noted on the low treatment plots during 1977, but their observation was not quantified in that study. Arbuscules are considered typical of young or new infections, while vesicles are viewed as representing the mature or reproductive state of the endophyte (Nicolson, 1959). The increase in vesicles and vesicles/arbuscule ratio may indicate an alternation of the normal symbiotic balance between the host grass and the endomycorrhizae in response to sulfur dioxide-induced stress.

The browning and senescence of roots was positively correlated with both sulfur content of leaves and roots ($p < .001$). Culm density is positively correlated ($p < .001$) with root sulfur but not sulfur in leaves.

Infection is negatively correlated with total relative root length ($p < .001$), browning and senescent roots ($p < .001$), and medium roots ($p < .01$), but positively correlated with large roots and root weight per culm, suggesting the endophyte is maintained at highest levels in stable *A. smithii* populations which have sufficient photosynthate to form larger root systems. Root enlargement is a function of cortex development and concurrent storage of photosynthate reserves in roots and rhizomes of this perennial grass where vegetative reproduction dominates. Declining infection levels and altered vesicle and arbuscule ratios may reflect limited photosynthate availability or altered host-plant metabolism rather than direct sulfur toxicity to the endomycorrhizae.

Vesicles decline with increasing total relative root length; arbuscules increase with total relative root length ($p < .05$), the frequency of small size roots ($p < .001$), and root weight per culm ($p < .001$), while the correlation between arbuscules and both browning and medium size roots is negative ($p < .001$). This appears to provide support for the concept that arbuscules are a sign of young infection found in association with active, functional root systems.

Total relative root length determined by point intersection is a reasonable index of root growth as is evidenced by its strong correlation with small roots, and negative correlation with both medium and large roots ($p < .001$) in these samples of fixed root weight (.5 g).

Browning and senescence is positively correlated with medium roots ($p < .01$), and high culm densities ($p < .001$), while being negatively correlated with small roots and high root weight per culm, again suggesting that root necrosis is greatest in plants with declining belowground growth vigor.

Small roots are positively correlated with root weight ($p < .01$) and culms per core ($p < .05$) in addition to their association with total relative root length and arbuscule frequency ($p < .001$). Medium roots are negatively correlated with root weight per culm ($p < .001$) but positively correlated with culms per core ($p < .05$).

DISCUSSION

The method of estimating the degree of host plant (*Agropyron smithii*) root infection resulting from the presence of benefical vesicular-arbuscular endomycorrhizae by measuring absorbance (400 NM) of root extracts from field populations is unreliable as employed. A significant positive correlation could not be established between absorbance and percent infection determined by microscopic inspection of 1978 root samples. This is at variance with the correlation observed for the 1977 work. Procedural changes from 1977 to 1978 may have resulted in absorbent adherence to filters, light degradation or alterations resulting from increased sample processing time. Phenolic compounds, released by dying roots, are a recognized interference with absorption determinations (Becker and Gerdemann, 1977). The proportion of browning and senescent roots increased strongly with sulfur dioxide treatment at ZAPS II and may have confounded absorbance readings. The absorbance method should be restricted to short-term potted plant trials until the biochemical nature of the absorbing compound is determined and more sensitive field plot methods develop.

The endomycorrhizal infection and the root dynamics of the two Off Plot Controls (OPC 1 and OPC 2) are similar in their distinction from the site II control (ZAPS IIA). They exhibit more extensive endomycorrhizal infection and lower culm density but decidedly more vigorous growth. The OPC exclosures have been maintained for 15 years. The ZAPS II exclosure was in place for the third year in 1978. The OPC communities should be stable in terms of release from grazing pressure while recovery at site II is still progressing. The ZAPS IIA plot also receives significant sulfur dioxide fumigation, with approximately 10 percent of the eight-minute median readings ranging from 2 to 6 pphm, Fumigations less than 2 pphm were below the detection limit of the analyzer (Preston *et al.*, Section 6). Sulfur dioxide treatment as low as 3.5 pphm for one to two hours has been demonstrated to decrease net photosynthesis and accelerate dark respiration in *Vicia faba* relative to clean air (Black and Unsworth, 1979). Prior to power plant operation or outside areas impacted by Colstrip power plant plume, the identified background level of sulfur dioxide in southeastern Montana is reported as approximately .1 pphm (Ludwick *et al.*, 1979). Thus, the possibility that A plot fumigation levels elicit physiological response in *A. smithii* should not be excluded. Tissue sulfurs on ZAPS IIA were not elevated relative to the OPC's in the July 1978 sample, although the 1977 data suggested that the A plots were accumulating ambient sulfur. Mean differences between the A plot and OPC's are likely to be small, and effects including accumulation are not a linear function of concentration x duration when fumigations are acute (Van Haut in Horsman *et al.*, 1979) in the sense of being relatively higher than background but of short duration. An answer to the question of elevated tissue sulfur derived from ambient concentrations on ZAPS IIA must await completion of the entire 1978 data base. Thus, differential grazing history and sulfur dioxide fumigation may contribute to the similar disparities of both OPC's relative to ZAPS IIA.

Although it does not integrate the consequences of acute fumigations well, the sulfur content of leaf tissue directly measures accumulated dosage. Leaf tissue sulfur means clearly segregate by plot, increasing with ambient level, on the intentionally fumigated ZAPS IIB, IIC and IID plots. However, individual leaf tissue sulfur variates within plots overlap substantially with those of other plots. The mean coefficient of variation for plots in the July collection was 16 percent. As is typical of living organisms, especially field populations, the biological responses are substantially more variable. Plot means do not segregate well except for the increase in browning and senescent roots. The biological responses determined in this study are certainly influenced by factors other than sulfur loading of tissues, but the correlation analysis estimates the degree of association between the biological responses and accumulated dosage without the additional variance of plot assignment.

The correlation between the decline in endomycorrhizal infection and increasing leaf tissue sulfur, which was also significant for the intentionally fumigated plots only, may reflect alterations in host plant metabolism rather than direct sulfur toxicity to the endophyte. Long-term sulfur deposition can lead to greater relative sulfur increases in belowground organs than in shoots (Rice *et al.*, 1979) and then chemical species metabolized from sulfur dioxide may cause direct toxicity. However, after fumigation for three growing seasons at ZAPS II, the increase in root sulfur is still small compared with that of tops, and mycorrhizal characteristics do not yet significantly correlate with root sulfur. Several workers have argued that sulfur dioxide and its higher oxidation states reduce photosynthetic electron transfer (Horsman and Welburn, 1976), and Ziegler (1975) has shown that ribulose - 1,5 - diphosphate carboxylase is inhibited by sulphite, reducing carbon dioxide binding. Sulfur dioxide-induced decreases in photosynthate availability in the root system of the host plant could alter the status of the fungal symbiont by decreasing this principle advantage it derives from the association. Total vesicles and the proportion of vesicles increase with leaf sulfur. The endophyte may be accelerating its entry into the sporulation stage or increasing its own stored food reserves as excess fixed carbon from the host declines (Hawker, 1966). The decline in total arbuscules and the proportion of arbuscules as leaf sulfur increases suggest the host plant is receiving a reduced mineral nutrition benefit from the endophyte. The vesicles/arbuscule ratio is positively correlated with leaf sulfur loading. The fungus may be diverting a larger proportion of its host-derived photosynthate from structures which directly benefit the host to those which maintain the endophyte. Harley (1959) has argued that the mycorrhizal relationship, although usually benefical, is potentially pathogenic. This is not to suggest that sulfur dioxide has completely shifted the host-fungus relationship from symbiosis to pathogency, but the symbiosis may be altered towards avirulence.

Potential changes in photosynthate flux to root systems are not the only changes in host biochemistry which could be affecting the host fungus balance. The observed increase in root necrosis with leaf and root tissue sulfur could be increasing phenolic releases to the rhizosphere. Phenols are typically involved in restricting fungal activity on roots (Ling-Lee *et al.*, 1977a, 1977b; Miles, 1968). A single biochemical mechanism is unlikely.

CONCLUSIONS

The following results were derived from the 1978 investigation of endomycorrhizae associated with *Agropyron smithii* (western wheatgrass) subjected to sulfur dioxide fumigation and provide significant confirmation of similar observations made in the 1977 study (Rice *et al.*, 1979).

(1) Sulfur content of leaf and root tissues increase with ambient sulfur dioxide treatment and leaf sulfur is positively correlated with root sulfur.

(2) The percentage of roots infected by endomycorrhizae decline with increasing leaf tissue sulfur.

(3) The number of vesicles increases with leaf sulfur content.

New significant findings were:

(1) Arbuscules decline with increasing leaf sulfur accumulation.

(2) The vesicles/arbuscule ratio increases with leaf sulfur load.

(3) The percentage of roots declining into a stage of browning and senescence increases with sulfur dioxide treatment.

In opposition to earlier results, a significant positive correlation could not be established between infection determined by visual scoring of roots and infection estimated from absorbance readings of root extracts. The colorimetric absorbance method as employed is unreliable for long-term studies involving field populations.

REFERENCES

Baylis, G. T. S. 1974. The Evolutionary Significance of Phycomycetous Mycorrhizas. In: Mechanisms of Regulation of Plant Growth, R. L. Bieleski, A. R. Ferguson, and M. M. Cresswell, eds. Bull. 12. The Royal Society of New Zealand, Wellington. pp. 191-193.

Becker, W. N., and J. W. Gerdemann. 1977. Colorimetric Quantification of Vesicular-Arbuscular Mycorrhizal Infection in Onion. New Phytol., 78:289-295.

Black, V. J., and M. H. Unsworth. 1979. Effects of Low Concentrations of Sulphur Dioxide on Net Photosynthesis and Dark Respiration of *Vicia faba*. J. Exp. Bot., 30(116):473-483.

Cox, G., and P. B. Tinker. 1976. Translocations and Transfer of Nutrients in Vesicular-Arbuscular Mycorrhizas. I. The Arbuscule and Phosphorous Transfer: A Quantitative Ultrastrutural Study. New Phytol., 77:371-378.

Dodd, J. L., W. K. Lauenroth, G. L. Thor, and M. B. Coughenour. 1979. Effects of Chronic Low Level SO_2 Exposure on Producers and Litter Dynamics. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 384-493.

Dunnett, C. W. 1955. A Multiple Comparison Procedure for Comparing Several Treatments with a Control. J. Amer. Stat. Assn., 50:1096-1121.

Gordon, C. C. 1976. Investigations of the Impact of Coal-Fired Power Plant Emissions Upon Plant Disease and Upon Plant-Fungus and Plant-Insect Systems. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana, R. A. Lewis, N. R. Glass, and A. S. Lefohn, eds. EPA 600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Or. pp. 61-90.

Granett, A. L. 1978. Effects on Mycorrhizal Fungi Associated with Tomato Plants Exposed to SO_2 or NO_2 Gas. Phytopath. News, 12(9):126.

Grzywacz, A. 1964. The Influence of Industrial Air Pollution on Pathological Fungi of Forest Trees. Sylwan., 155:55-62.

Harley, J. L. 1959. The Biology of Mycorrhiza. Interscience Publications, Inc., New York. 233 pp.

Hawker, L. E. 1966. Environmental Influences on Reproduction. In: The Fungi. Volume II. The Fungal Organisms, G. C. Ainsworth and A. S. Sussman, eds. Academic Press, New York. pp. 435-469.

Heagle, A. S. 1973. Interactions Between Air Pollutants and Plant Parasites. Ann. Rev. Phytopath., 11:365-388.

Horsman, D. C., T. M. Roberts, M. Lambert, and A. D. Bradshaw. 1979. Studies on the Effect of Sulphur Dioxide on Perennial Ryegrass (*Lolium perenne* L.). I. Characteristics of Fumigation System and Preliminary Experiments. J. Exp. Bot., 30(116):485-493.

Horsman, D. C., and A. R. Wellburn. 1976. Appendix II. Guide to the Metabolic and Biochemical Effects of Air Pollutants on Higher Plants. In: Effects of Air Pollutants on Plants, T. A. Mansfield, ed. Cambridge University Press, Cambridge. pp. 185-199.

Kinden, D. A., and M. F. Brown. 1975. Electron Microscopy of Vesicular-Arbuscular Mycorrhizae of Yellow Poplar. II. Intracellular Hyphae and Vesicles. Can J. Microbiol., 21:1768-1780.

Ling-Lee, M., A. E. Ashford, and G. A. Chilvers. 1977a. A Histochemical Study of Phenolic Materials in Mycorrhizal and Uninfected Roots of *Eucalyptus fastigata* Deane and Maiden. New Phytol., 78:313-318.

Ling-Lee, M., A. E. Ashford, and G. A. Chilvers. 1977b. A Histochemical Study of Polysaccharide Distribution in Eucalyptus Mycorrhizas. New Phytol., 80:135-141.

Ludwick, J. D., D. B. Weber, K. B. Olsen, and S. R. Garcia. 1979. Air Quality Measurements in the Coal-Fired Power Plant Environment of Colstrip, Montana. PNL-2964, UC90i, Battelle Pacific Northwest Laboratory, Richland, Wa. 42 pp.

McCool, P. M., J. A. Menge, and O. C. Taylor. 1979. Effects of Ozone and HCl Gas on the Development of the Mycorrhizal Fungus *Glomus fasciculatus* and Growth of 'Troyer' Citrange. J. Amer. Soc. Hort. Sci., 104(2):151-154.

Miles, P. W. 1968. Insect Secretations in Plants. Ann. Rev. Phytopath., 6:137-164.

Newman, E. I. 1966. A Method of Estimating the Total Length of Root in a Sample. J. Appl. Ecol., 3:139-145.

Nicolson, T. H. 1959. Mycorrhizae in the Gramineae. I. Vesicular-Arbuscular Endophytes, with Special Reference to the External Phase. Trans. Brit. Mycol. Soc., 42:421-438.

Preston, E. M., and T. L. Gullett. 1979. Spatial Variation of Sulfur Dioxide Concentrations on ZAPS During the 1977 Field Season. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 306-330.

Rice, P. M., L. H. Pye, R. Boldi, J. O'Loughlin, P. C. Tourangeau, and C. C. Gordon. 1979. The Effects of "Low Level SO_2 Exposure on Sulfur Accumulation and Various Plant Life Responses of Some Major Grassland Species on the ZAPS Sites. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 494-591.

Richards, B. N., and G. K. Voigt. 1964. Role of Mycorrhiza in Nitrogen Fixation. Nature, 201:310-311.

Ruehle, J. L., and D. H. Marx. 1979. Fiber, Food, Fuel and Fungal Symbionts. Science, 206(4417):419-422.

Safir, G. R., J. S. Boyer, and J. W. Gerdemann. 1971. Mycorrhizal Enhancement of Water Transport in Soybean. Science, 172:581-583.

Sanders, F. E., and P. B. Tinker. 1973. Phosphate Flows into Mycorrhizal Roots. Pest. Sci., 4:385.

Sobotka, A. 1964. Effects of Industrial Emissions on Soil Biology of Norway Spruce Stands in the Ore Mountains. Lesnicky Casopis, 10:987-1002.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, San Francisco, Ca. 776+ pp.

Sparling, G. P., and P. B. Tinker. 1975. Mycorrhizas in Penine Grassland. In: Endomycorrhizas, F. E. Sanders, B. Mosse, and B. B. Tinker, eds. Academic Press, London. pp. 545-560.

Van Haut, H. 1961. Staub., 21:52-56.

Zak, B. 1964. Role of Mycorrhizae in Root Disease. Ann. Rev. Phytopath., 2:377-392.

Ziegler, I. 1975. The Effect of SO_2 Pollution on Plant Metabolism. Residue Reviews--Residues of Pesticides and Other Contaminants in the Total Environment, 56:79-105.

SECTION 9

RESPONSES OF TWO LICHEN SPECIES TO LOW-LEVEL SO_2

S. Eversman

ABSTRACT

Usnea hirta (L.) Wigg. and *Parmelia chlorochroa* Tayl. exhibited reduced photosynthesis rate, disrupted chloroplast structure, and increased algal plasmolysis in 27-92 days in ZAPS I and II plot B. Differences in respiration rates and relative chlorophyll extract absorbances were not significant among control *U. hirta* samples and *U. hirta* samples from fumigation plots A and B.

INTRODUCTION

The ZAPS (Zonal Air Pollution System) SO_2 fumigation systems were designed to investigate effects of low-level chronic SO_2 stress on grassland ecosystem components. Two lichen species, *Usnea hirta* (L.) Wigg, and *Parmelia chlorochroa* Tayl., have been transplanted into each ZAPS treatment plot each spring. Samples have been collected monthly and analyzed for anatomical and physiological injuries due to SO_2 stress.

Responses in the lichens collected from medium and high treatment plots have been acute and well-defined: significantly reduced respiration rates and relative chlorophyll extract absorbance, and significantly increased algal plasmolysis ($P < .05$) within 30-60 days in these plots (Eversman, 1978). In the low treatment it has been somewhat difficult to precisely identify injuries in lichen samples that are definitely attributable to SO_2 effects. The primary purpose of the 1978 field season activities was to better distinguish between transplant and SO_2 effects, and possible combinations of the two.

MATERIALS AND METHODS

Ponderosa pine branches containing *U. hirta* were transplanted onto one fencepost in each ZAPS plot. Posts were in the northeast portion of each plot adjacent to EPA sulfation plates. Three vertical levels of branches were on each post corresponding to the sulfation plates: 15, 35, and 70 cm above the ground. *Parmelia chlorochroa* thalli were tied onto the branches adjacent *U. hirta* thalli.

Transplants were made May 3, June 6, and July 6, 1978. Collections for analysis were made August 3 from each transplant, and from controls.

Respiration rates were determined manometrically. Algal plasmolysis was determined with a light microscope. Chlorophyll extracts were made with hot methanol, and read at 665 nm in a spectrophotometer. These methods have been described previously (Eversman, 1977, 1978).

Relative photosynthetic rates were determined by exposing a small piece of thallus (*ca.* 0.3 mm^2) to $^{14}CO_2$ for 20 seconds in a field porometer (Shimshi, 1969; Moser and Nash, 1978). The lichen fragments were combusted, CO_2 was collected in trapping fluids, and the radioactivity was determined with a scintillation counter.

Preparation for transmission electron microscopy included fixation with glutaraldehyde-osmium tetroxide, embedding in Spurr's resin, and post-staining with uranyl acetate and lead citrate. A Phillips 201 transmission electron microscope at Arizona State University was used for electron-micrographs. All lichen tissue from control, plot A and plot B samples was taken from green, non-bleached thallus tips.

RESULTS AND DISCUSSION

Differences in respiration rates between *U. hirta* samples in plots A and B were not significant after 92 days of SO_2 treatment (Figure 9.1). These results are similar to those obtained in previous years (Eversman, 1978). Respiration rates were not determined for *P. chlorochroa* in the ZAPS sites in 1978.

Algal plasmolysis percentages were significantly higher (non-overlapping 0.95 confidence intervals) in *U. hirta* and *P. chlorochroa* samples from ZAPS I, plot B, than in control samples (Figure 9.2). This tissue analysis has given the most consistent significant differences between plot A and plot B samples.

Because of large amounts of material required, chlorophyll extract absorbances were not determined for *P. chlorochroa* from ZAPS sites. Differences in *U. hirta* samples from plots A and B were not distinct (Table 9.1). Relative chlorophyll extract absorbance values were generally lower in samples from the ZAPS sites than in samples from native habitats or from

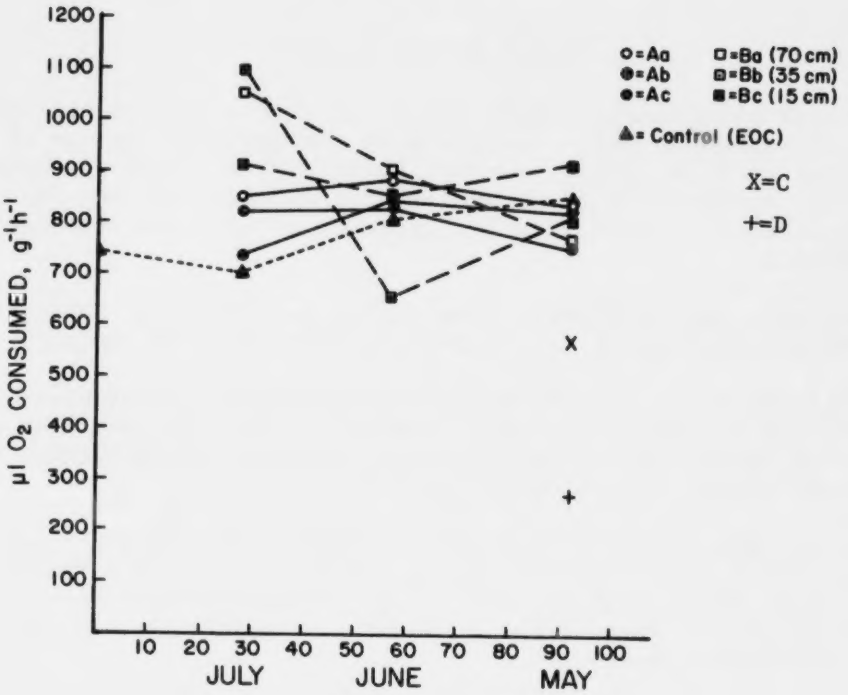

Figure 9.1. Respiration rates of *U. hirta* from ZAPS I plots and control samples (EOC = Site P10, in Section 3, Figure 3.1.). Values are means of three samples expressed as μl oxygen consumed $g^{-1}h^{-1}$. Abscissa = days SO_2.

transplant sites in the Colstrip area (Section 3). It is not yet clear if differences are due entirely to SO_2 or to some sun bleaching since the ZAPS sites are more exposed than the ponderosa pine forests from which *U. hirta* was transplanted.

Mean relative photosynthesis rates were less in samples from plot B than in samples from plot A (Figure 9.3). Comparisons with control sites P10 (EOC, transplant source) and P11 (SEAM 1) were inconclusive. Large variances about the means resulted in no statistical differences between any of the plot A, plot B, or control samples. Photosynthesis rates in plot C were nearly zero, and they were zero for samples of both species from plot D.

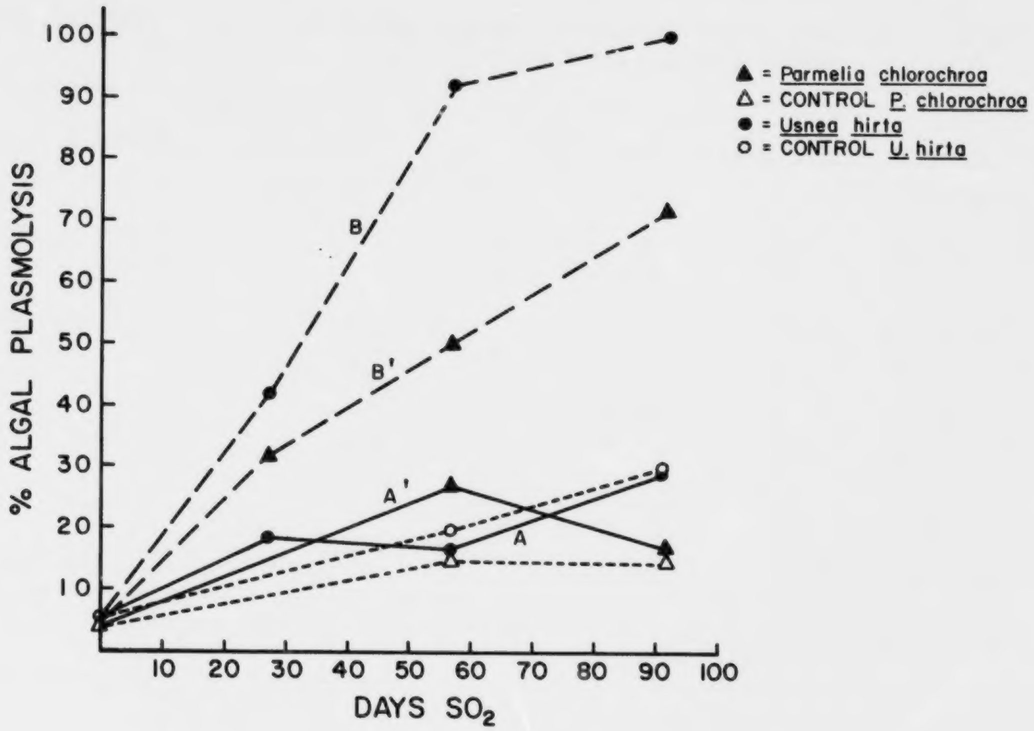

Figure 9.2. Percentage of algal plasmolysis in *U. hirta* and *P. chlorochroa* in ZAPS I and II, plots A and B. A, B = ZAPS I. A', B' = ZAPS II. Control samples are from transplant sources (Sites P10, G7).

Electron Microscopy

Figures 9.4 and 9.8 illustrate control samples of *U. hirta* and *P. chlorochroa,* respectively. Large chloroplasts and small amounts of cytoplasm are present (Chervin, *et al.,* 1968; Peveling, 1973). Exposure to SO_2 on the ZAPS plots resulted in increasing plasmolysis and internal disorganization: thylakoid swelling, visible in Figures 9.5, 9.6, and 9.9. Starch grains in Figures 9.5 and 9.6 may be due to transplant effects on humidity (Ascaso, 1978), light differences, or SO_2 (Ikonen and Karenlampi, 1967). It is not yet clear which factors are causing these changes in the chloroplasts.

Slight bleaching was present in the samples from plot B although only green thallus tips were used for electron microscopy. Not all the algal cells exhibited fine structural injury or starch grains visible in the chloroplasts as in Figures 9.5 and 9.6; some were nearly identical with control cells. Some intact and some injured cells would be expected when compared with plasmolysis, chlorophyll extract, and photosynthesis observations with similar samples. An increased number of starch grains may

Table 9.1. RELATIVE CHLOROPHYLL EXTRACT ABSORBANCE VALUES (665 nm) FOR *USNEA HIRTA*, ZAPS I and II, 1978.

	June			August	
	Days SO_2	Mean ± 0.95 CI		Days SO_2	Mean ± 0.95 CI
Control (EOC)	0	0.630 ± 0.493	Control (EOC)	0	1.24 ± 0.132
II Aa	30	0.213 ± 0.102	I Aa	27	0.365 ± 0.221
II Ba	30	0.173 ± 0.069	I Bb	27	0.445 ± 0.181
II Ab	30	0.793 ± 0.791	I Aa	57	0.673 ± 0.544
II Bb	30	0.352 ± 0.162	I Bb	57	0.475 ± 0.587
II Ac	30	0.586 ± 0.096	I Aa	92	0.241 ± 0.336
II Bc	30	0.280 ± 0.037	I Ba	92	0.310 ± 0.233
I Ac	30	0.552 ± 0.291			
I Bc	30	0.590 ± 0.424			

Pairs are matched according to height above ground and days of SO_2 exposure. a = 70 cm, b = 35 cm, c = 15 cm above ground. Values are given as means of three samples ± 0.95 confidence intervals. Confidence intervals = $t_{.05}$ x standard error/ n for each mean.

explain respiration rates considerably higher than control rates. More detailed comparisons are currently being undertaken with control samples, other transplants, plot A, and plot B samples to distinguish transplant and SO_2 effects. Preliminary electronmicrographs of samples from plot A and off-ZAPS-plot grassland transplant samples showed less disturbance of chloroplast and cytoplasm, and fewer starch grains than in samples from ZAPS plot B. These results are being examined further.

All samples from ZAPS plots C and D have been bleached yellow in 30-60 days of exposure to the medium and higher levels of SO_2. The complete loss of protoplasmic structure visible in Figure 9.7 is presumably caused by SO_2. The reduced respiration and photosynthesis rates and disordered internal structure are probably related.

CONCLUSIONS

The levels of SO_2 in ZAPS plots C and D have been high enough to cause acute visible and physiological tissue damage in *U. hirta* and *P. chlorochroa*. Samples from ZAPS plot B (both I and II) are visibly more bleached than samples from ZAPS A and samples collected in their native habitats. This indicates definite SO_2 injury to the lichens in plot B. The most sensitive methods of defining the amount of damage appear to be examining the algal cell condition (plasmolysis), bleaching of the thallus, and observing the fine structure with transmission electron microscopy. Respiration rates and chlorophyll extract absorbance have been used, and will continue to be used, but appear to be less sensitive techniques. Respiration is a measure of the thallus as a whole, which is mostly fungal tissue and presumably not as sensitive as algal tissue.

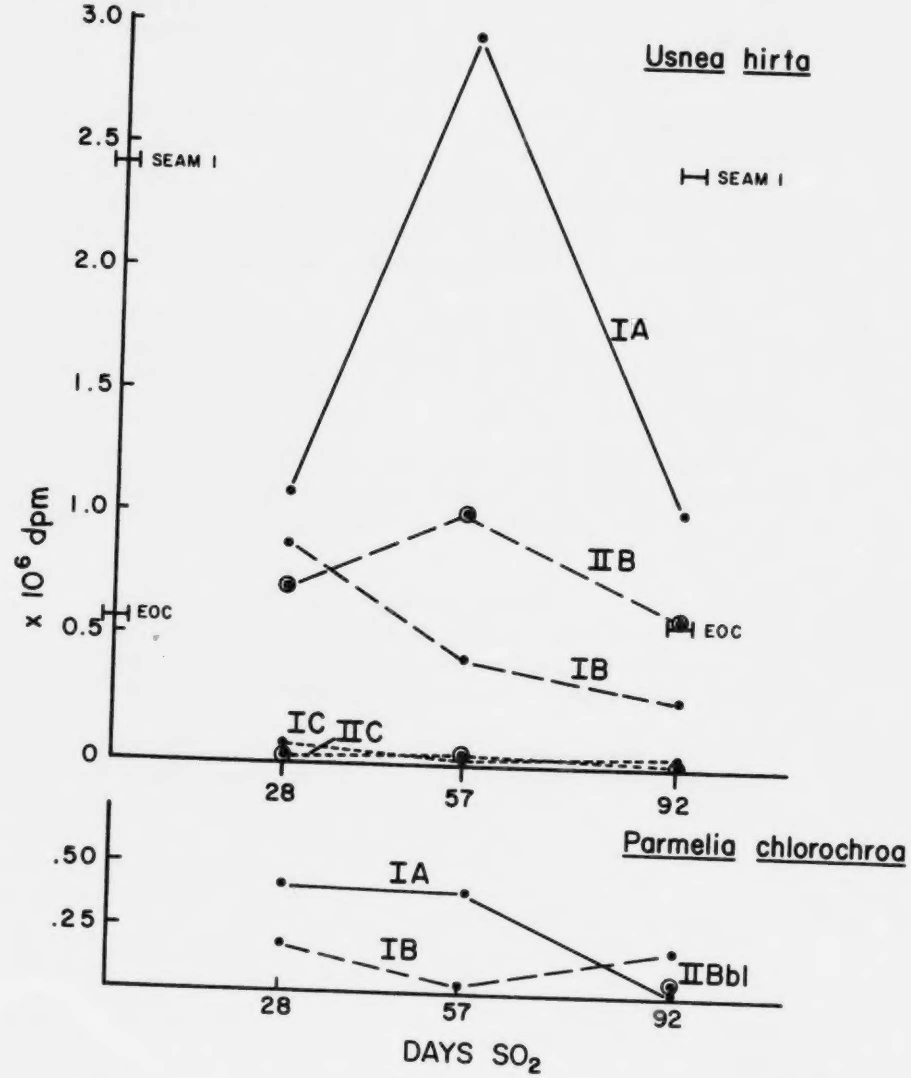

Figure 9.3. Relative photosynthesis rates of *U. hirta* and *P. chlorochroa* from ZAPS I and II. Rates are expressed as means of three samples (decays per minute). EOC (Site P10) and SEAM I (Site P11) were controls.

FIGURE CAPTIONS

Figure 9.4. Algal cell of *Usnea hirta* from Site P10. W = cell wall. C = chloroplast with thylakoids. Cy = cytoplasm. P = pyrenoid in chloroplast with dark bodies assumed to be lipids. Magnification 34,250X.

Figure 9.5. Algal cell of *U. hirta* after 27 days SO_2 in ZAPS I plot B. Increased plasmolysis and starch grains (S) in chloroplast are visible. N = nucleus with nucleolus. F = fungal hypha. Lipid bodies in this electronmicrograph stain light-colored; they are dark in the cytoplasm in Figure 11.4. This is not a treatment-related phenomenon. Magnification 14,000X.

Figure 9.6. *U. hirta* algal cells after 27 days in ZAPS I plot B. The thylakoids in the cell on the left are swollen; the cytoplasm in the cell on the right has become denser and disorganized. Magnification 40,391X.

Figure 9.7. *U. hirta* algal cell after 92 days in ZAPS I plot C. No internal structure is distinguishable. All algal cells are bleached and plasmolyzed in the lichen thallus. Magnification 38,400X.

Figure 9.8. Algal cell of *Parmelia chlorochroa* control sample. P = pyrenoid with dark lipid bodies. L = light-staining lipid bodies in cytoplasm. ER = rough endoplasmic reticulum. F = fungal hypha. M = mitochondrion. Magnification 21,000X.

Figure 9.9. *P. chlorochroa* algal cell after 28 days in ZAPS I plot B. Thylakoids in chloroplast are disrupted. Magnification 41,100X.

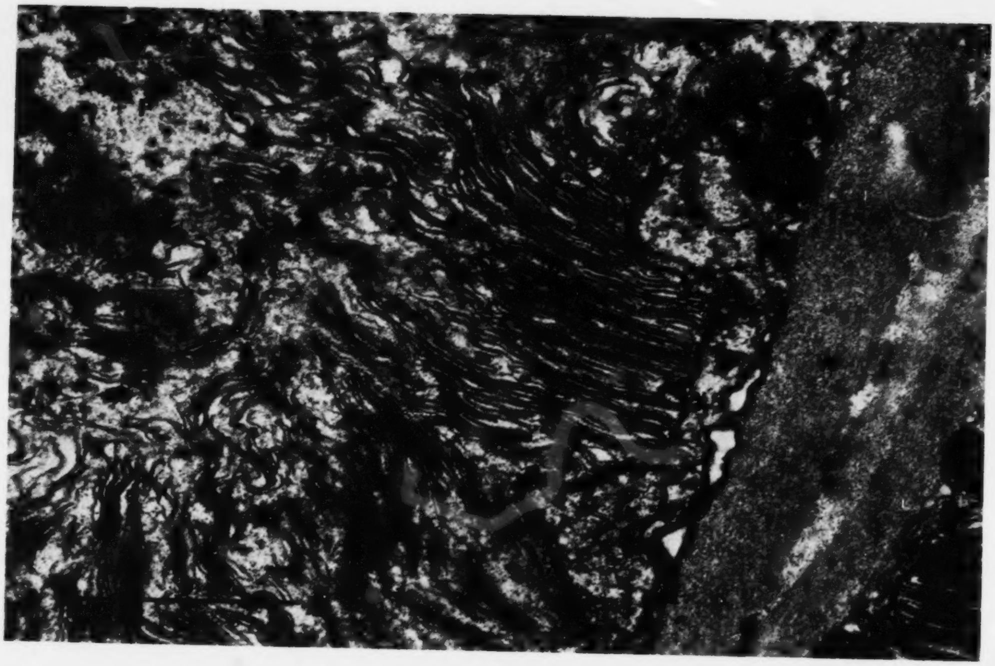

Figure 9.4

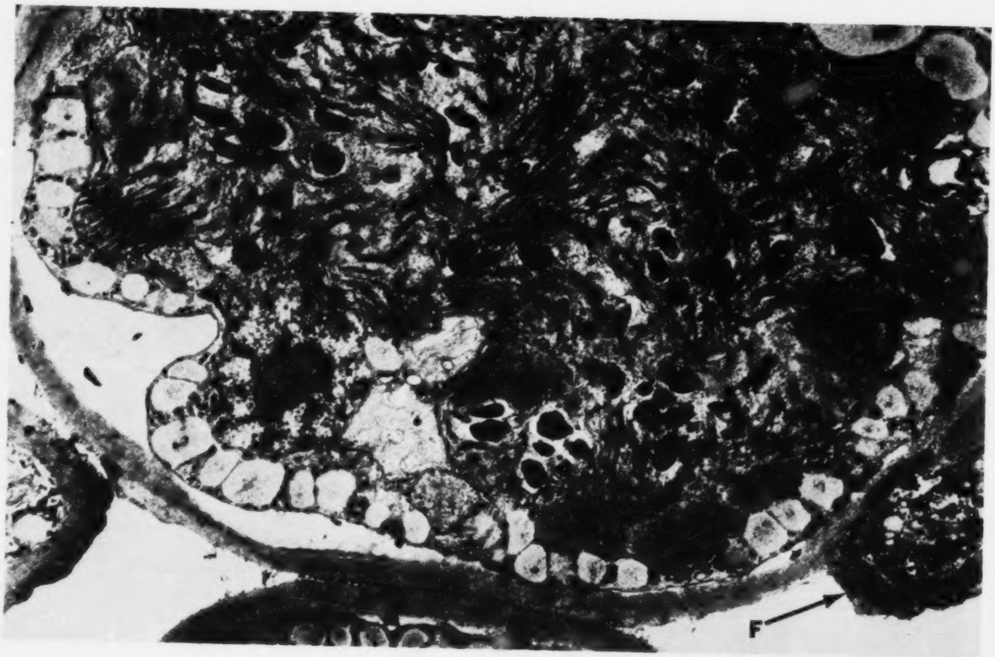

Figure 9.5

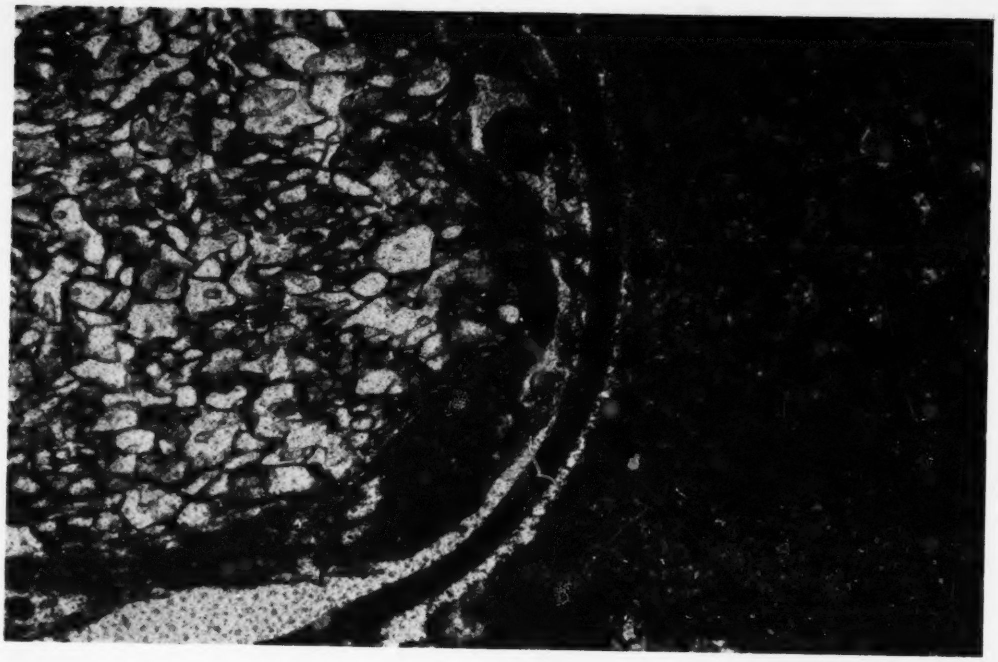

Figure 9.6.

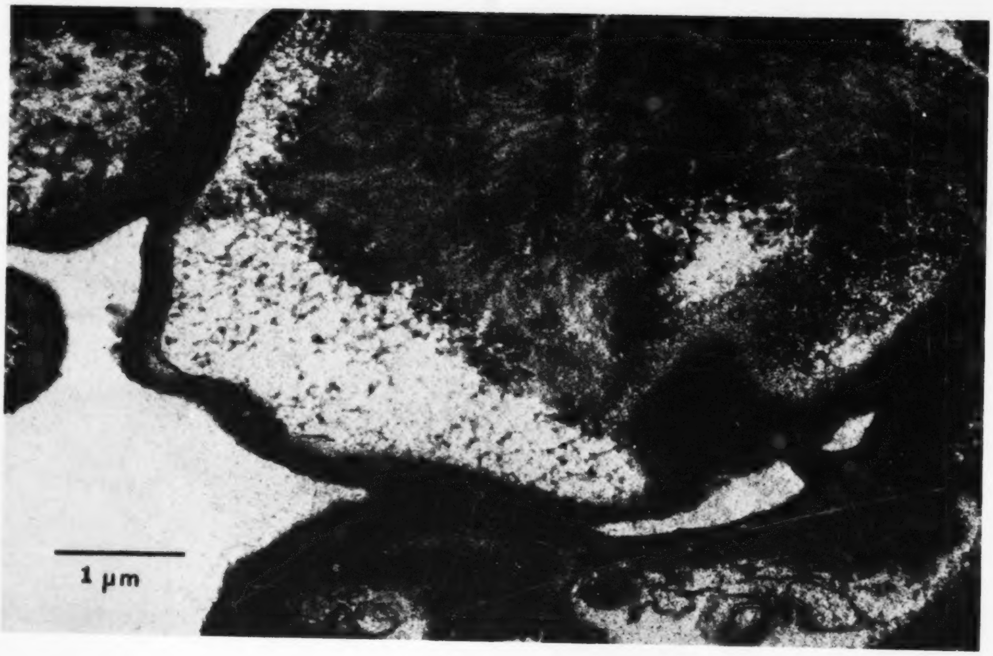

Figure 9.7.

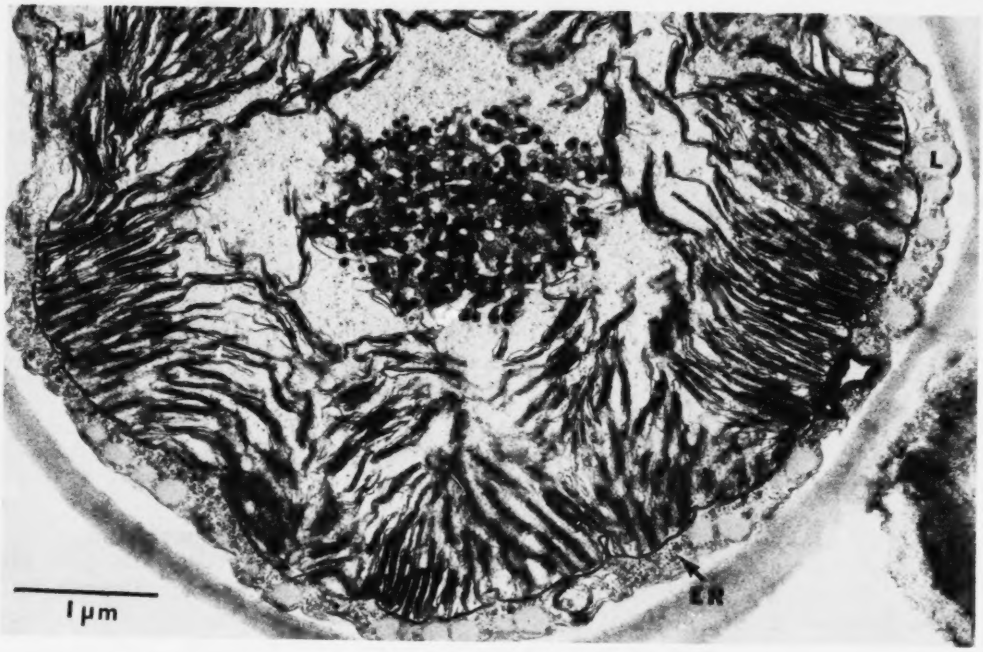

Figure 9.8.

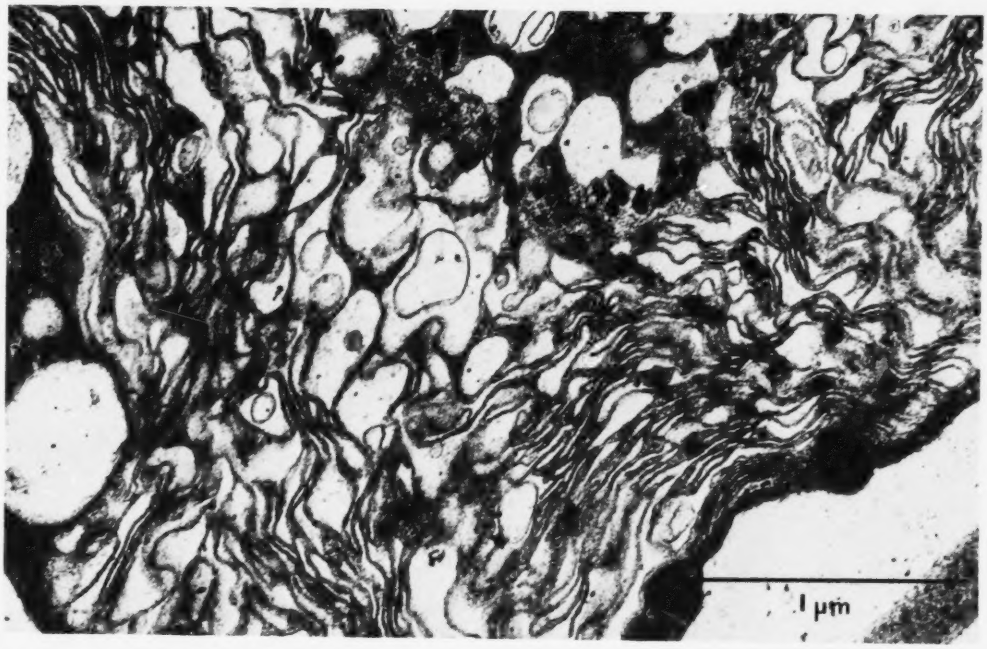

Figure 9.9.

REFERENCES

Ascaso, C. 1978. Ultrastructural Modifications in Lichens Induced by Environmental Humidity. Lichenologist 10: 209-219.

Chervin, R. E., G. E. Baker, and H. R. Hohl. 1968. The Ultrastructure of Phycobiont and Mycobiont in Two Species of *Usnea*. Can. J. Bot. 46: 241-245.

Eversman, S. 1977. Effects of Low-Level SO_2 Stress on Two Lichen Species. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana. EPA 600/3-78-021. Environmental Protection Agency, Corvallis. pp. 385-398.

Eversman, S. 1978. Effects of Low-Level SO_2 on *Usnea hirta* and *Parmelia chlorochroa*. Bryologist 81(3):368-377.

Ikonen, S. and L, Karenlampi. 1967. Physiological and Structural Changes in Reindeer Lichens Transplanted Around a Sulphite Pulp Mill. In: Proceedings of the Kuopio Meeting on Plant Damages Caused by Air Pollution, L. Karenlampi, ed. Kuopio.

Moser, T. J. and T. H. Nash III. 1978. Photosynthetic Patterns of *Cetraria cucullata* (Bell.) Ach. at Anaktuvuk Pass, Alaska. Oecologia (Berl.) 34: 37-43.

Peveling, E. 1973. Fine Structure of Lichens. In: The Lichens, Ahmadjian, V. and M. Hale, eds. Academic Press, New York. pp. 147-184.

Shimshi, D. 1969. A Rapid Field Method for Measuring Photosynthesis with Labelled Carbon Dioxide. J. Expt. Bot. 20: 381-401.

SECTION 10

SULFUR ACCUMULATION IN WESTERN WHEATGRASS EXPOSED TO CONTROLLED SO_2 CONCENTRATIONS

W. K. Lauenroth, C. J. Bicak, and J. L. Dodd

ABSTRACT

Sulfur concentrations of western wheatgrass tillers and individual leaves were measured from plants exposed to four SO_2 concentrations on ZAPS. Sulfur concentration of plants was a linear function of either time of exposure or concentration. Young leaves and the youngest portion of leaves contained less sulfur than their older counterparts regardless of whether or not they had been exposed to SO_2. Current hypotheses that relate plant sensitivity to amount of sulfur taken up do not apply for western wheatgrass.

INTRODUCTION

An increase in the plant tissue concentration of sulfur often serves as an indication of sulfur dioxide exposure. Exposures to high concentrations of sulfur dioxide often result in low sulfur uptake because of irreversible damage to the stomatal mechanisms. In contrast, low sulfur dioxide concentrations can result in the accumulation of large amounts of sulfur (Guderian, 1977). Even in severely polluted areas, low atmospheric concentrations of SO_2 predominate levels that comprise the measurable SO_2 concentrations. Consequently, the accumulation of sulfur in plant organs is an easily determined impact of SO_2 pollution. The physiological importance of increased sulfur concentrations and the balance between positive and negative effects will depend upon the particular species and its rate of growth and metabolism. Cowling and Locker (1978) demonstrated that low concentrations of SO_2 (approximately 2 pphm) can correct sulfur deficiency in perennial ryegrass (*Lolium perenne* L.)CV S23. In contrast, Bell and Clouth (1973) reported a 50 per cent reduction in the yield of the same species at a concentration of approximately 7 pphm.

Recently it has been hypothesized that plant sensitivity to SO_2 is dependent, in part, upon rate of SO_2 uptake. Garsed and Read (1977) interpreted the total quantity of SO_2 taken up by different plant species under identical exposures as an indication of the plant's capacities to avoid the deleterious effects of SO_2. Klein *et al.* (1978) and Kondo and Sugahara (1978) found that plant species known to be resistant took up smaller quantities of SO_2 than plants known to be susceptible, where sensitive species are characterized by visible injury criteria.

Sulfur accumulation rates for western wheatgrass (*Agropyron smithii* Rydb.), a species considered resistant to SO_2 using visible injury criteria (Heitschmidt *et al.*,1978), are reported in this paper. The data were collected from both ZAPS I and ZAPS II.

MATERIALS AND METHODS

Plant Analysis

Western wheatgrass plants consist of a number of individual tillers connected by a rhizome located at a depth of approximately 5-10 cm in the soil. Sulfur content of entire plants was assessed by collecting a large number of individual live tillers from each SO_2 treatment approximately once each month during the growing season. Small amounts of dead tissue associated with each tiller were also included in the analyses. Each sulfur value reported is the mean of two samples. Sulfur content was measured with a Leco Induction Furnace (Laboratory Equipment Corporation, St. Joseph, Michigan, USA) with the inorganic sulfate ion $(SO_4=)^2$ the primary form of sulfur recovered.

The sulfur content of western wheatgrass leaves was assessed by collecting the oldest and youngest fully expanded green leaf from a large number of tillers in the control and high concentration plots in late June 1977. Each leaf was further separated into a younger and older half to investigate gradients in sulfur content in the same leaf. Older leaves are located at the base of the plant, while younger leaves are near the top. The oldest portion of the leaf is near the tip, while the youngest portion is near the base. Triplicate samples of each leaf and age were analyzed for sulfur content.

RESULTS AND DISCUSSION

Sulfur content of western wheatgrass plants was positively correlated with both time of exposure (Table 10.1) and concentration of SO_2 (Table 10.2). Sulfur accumulation rates for the May through August growing periods in the medium treatment ranged from 193-460 $\mu g \cdot g^{-1} \cdot month^{-1}$. Corresponding values for the high concentration treatment ranged from 385-650 $\mu g \cdot g^{-1} \cdot month^{-1}$. Accumulation of sulfur by plants in the low treatment was very weakly related to time of exposure and as expected the slopes of the regression lines were much smaller than those for plants from either the medium or high treatments. The differences in the accumulation rates among years as illustrated by the slopes of the regressions (Table 10.1) may be at least partially explained by differences in growing season precipitation which largely controls growth rate and productivity of these grasslands. Since sulfur is accumulated both from the soil solution and the atmosphere when SO_2 is present, the concentration measured within the plant will be determined by the growth rate, the duration of favorable growing conditions, the concentration of SO_2, the duration of

TABLE 10.1. LINEAR REGRESSION PARAMETERS FOR THE RELATIONSHIP OF SULFUR ACCUMULATION IN *AGROPYRON SMITHII* AND TIME OF EXPOSURE TO THREE SO_2 CONCENTRATIONS AT TWO SITES IN THREE GROWING SEASONS

Treatment	Year	Site	Y intercept*	Slope†	r2
Low	1975	I	1350	0	0
	1976	I	1000	120	0.90
	1976	II	1300	50	0.09
	1977	I	1388	18	0.03
	1977	II	1523	37	0.02
Medium	1975	I	1150	390	0.90
	1976	I	1200	400	0.92
	1976	II	1050	460	0.86
	1977	I	1487	237	0.85
	1977	II	1607	193	0.72
High	1975	I	800	650	0.96
	1976	I	1450	620	0.92
	1976	II	2550	560	0.98
	1977	I	1668	385	0.85
	1977	II	2458	441	0.74

* $\mu g \cdot g^{-1}$

† $\mu g \cdot g^{-1} \cdot month^{-1}$

TABLE 10.2. LINEAR REGRESSION PARAMETERS FOR THE RELATIONSHIP OF SULFUR CONTENT IN WESTERN WHEATGRASS EXPOSED TO THREE SO_2 TREATMENTS AND CONCENTRATIONS

Site	Year	Y intercept*	Slope†	r2
I	1975	1110	185	0.95
	1976	835	277	0.98
	1977	1123	208	0.97
II	1976	799	370	0.94
	1977	961	277	0.96

* $\mu g \cdot g^{-1}$

† $\mu g \cdot pphm^{-1}$

exposure, and the concentration in the soil sulfur pool. In both 1975 and 1976 precipitation amounts and distributions favored sulfur uptake compared to 1977.

Sulfur accumulation was also linearly related to treatment concentration. Average sulfur contents of the control plants ranged from 800 to 1100 $\mu g \cdot g^{-1}$. Thomas *et al.* (1950) surveyed 29 trees, shrubs, and herbs throughout the western United States and found total sulfur contents ranging from 400 $\mu g \cdot g^{-1}$ in conifer needles to 35100 $\mu g \cdot g^{-1}$ in a halophytic herb (*Sueda* spp.). Sulfur accumulation rates increased in the presence of SO_2 at the rate of 185 to 370 $g \cdot g^{-1}$ for each pphm of SO_2 to which the plants were exposed during the growing season. The largest differences in incremental accumulation rates occurred between sites. At Site I the rates varied by 92 $g \cdot g^{-1} \cdot$ pphm and indicated no relationship to the wet years (1975 and 1976). Rates were higher at Site II but demonstrated the same trend between years in that 1976 had the largest rate.

Thomas *et al.* (1950) hypothesized that after entry into the leaf, SO_2 is rapidly dissolved on most surfaces to form sulfite, which is then slowly oxidized to sulfate. Thomas and Hendricks (1956) found sulfate was much less toxic to plants than sulfite. It has been assummed that this oxidation step was an important determinant of whether a plant would be damaged by a given exposure to SO_2. However, Malhotra and Hocking (1976) have found that toxic levels of sulfate are eventually attained, leading to injury symptoms. This explanation was used by Heitschmidt *et al.* (1978) to explain increased leaf senescence in western wheatgrass as a result of low-concentration exposures. Because senescence begins at the leaf tip and spreads downward, this implies that sulfate concentrations accumulate with the same pattern. The data in Table 10.3 verify that there are substantial gradients between the tips and bases of individual leaves and between leaves of different ages. Jäger (1976) reported the same trends in spruce needles exposed to SO_2. In addition, he

TABLE 10.3. SULFUR CONTENTS* OF TWO DIFFERENT LEAF AND TISSUE AGES OF PLANTS GROWING IN THE CONTROL AND HIGH-CONCENTRATION TREATMENT PLOTS

	Leaf age			
	Young		Old	
Treatment	Base	Tip	Base	Tip
Control	830 ± 60	1117 ± 25	980 ± 60	1197 ± 95
High	1810 ± 121	2643 ± 186	3883 ± 6	5010 ± 144

* $\bar{X}$ ± SD in $\mu g \cdot g^{-1}$

found gradients between tips and bases of control plants, which agrees with our results. Guderian (1977) reported that secondary transport of sulfur taken up as SO_2 results in highest concentrations in leaf tips and margins. Both Jäger (1976) and Guderian (1977) cite Halbwachs (1963) to explain that this transport is affected by sulfate moving with water and the existence of a suction gradient favoring movement towards leaf tips and margins.

CONCLUSIONS

The degree of SO_2 uptake has been suggested as an important factor in determining sensitivity (Garsed and Read, 1977; Klein *et al.*, 1978). The limitations imposed by so simplistic an explanation relate largely to the meaning of sensitivity and its interactions with dose. The often-used synonymy of sensitivity and visible leaf injury places restrictions upon interpretations of responses to low-concentration exposures that may result in important physiological responses with no visible injury. Western wheatgrass is shown to accumulate significant quantities of sulfur when exposed to SO_2, yet we have never found symptoms of SO_2 injury to leaves. We have found increased leaf senescence (Heitschmidt *et al.*, 1978) and chlorophyll destruction (Lauenroth and Dodd, in prep.) as a result of exposures to low-SO_2 concentrations.

REFERENCES

Bell, J. S., and W. S. Clough. 1973. Depression of Yield in Ryegrass Exposed to Sulphur Dioxide. Nature (Lond.), 241:47-49.

Cowling, D. W., and D. R. Lockyer. 1978. The Effect of SO_2 on *Lolium perenne* L. Grown at Different Levels of Sulphur and Nitrogen Nutrition. J. Exp. Bot., 29:257-265.

Garsed, S. G., and D. J. Read. 1977. The Uptake and Metabolism of $^{35}SO_2$ in Plants of Differing Sensitivity to Sulphur Dioxide. Environ. Pollut., 13:173-186.

Guderian, R. 1977. Air Pollution. Springer-Verlag, Berlin. 127 pp.

Halbwachs, G. 1963. Utersuchnunger über gerichete aktive strömungen and stofftransporte in blatt. Flora, 153:333-357.

Heitschmidt, R. K., W. K. Lauenroth, and J. L. Dodd. 1978. Effects of Controlled Sulphur Dioxide Fumigation on Western Wheatgrass in a Southeastern Montana Grassland. J. Appl. Ecol., 14:693-702.

Jäger, H. J. 1976. S-Lokalisation in SO_2-begasten Fichtennadeln. Eur. J. For. Pathol., 6:25-29.

Klein, H., H. J. Jäger, W. Domes, and C. H. Wong. 1978. Mechanisms Contributing to Differential Sensitivities of Plants to SO_2. Oecologia, 33:203-208.

Kondo, N., and K. Sugahara. 1978. Changes in Transpiration Rate of SO_2 Fumigation and the Participation of Abscisic Acid. Plant Cell Physiol., 19:365-373.

Lauenroth, W. K., and J. L. Dodd. (In prep.) Chlorophyll Reduction in Western Wheatgrass Exposed to Sulfur Dioxide.

Malhotra, S. S., and D. Hocking. 1976. Biochemical and Cytological Effects of Sulphur Dioxide on Plant Metabolism. New Phytol., 76:227-237.

Thomas, M. D., and R. H. Hendricks. 1956. Effects of Air Pollution on Plants, In: Air Pollution Handbook, P. L. Magill, F. Holden and C. Ackley, eds. McGraw-Hill Book Co., Inc., New York. pp. 9.1-9.44.

Thomas, M. D., R. H. Hendricks, and G. R. Hill. 1950. Sulfur Metabolism of Plants. Ind. Eng. Chem., 42:2231-2235.

SECTION 11

WEIGHT AND GERMINATION RESPONSES OF GRASS SEEDS FROM PARENTAL STOCK SUBJECTED TO SULFUR DIOXIDE FUMIGATION

P. M. Rice, C. C. Gordon, and P. C. Tourangeau

ABSTRACT

In 1977, seeds were collected from three perennial grass species and one weedy biennial forb which had been subjected to chronic sulfur dioxide fumigation at ZAPS I and II. These seeds were then incubated without further sulfur dioxide treatment in growth chambers to assess the consequences of stressing the parental stock on various germination responses and seed weight. Edaphic factors at the site I control (ZAPS IA) may be obscuring potential treatment effects. Results from site II provide evidence of a depression of seed responses in three of the four species studied. Responses do not change incrementally with changes in fumigation levels, thus other factors or experimental procedures may be influencing experimental results. Germination trials to be conducted on 1978-79 seed collections will incorporate procedural alterations to reduce the potential effect of these other factors.

INTRODUCTION

Community structure and composition can be altered by air pollution-induced stress (Winner and Bewley, 1978; Farrar *et al.*, 1977; Westwood, 1979) at ambient concentrations which do not directly cause mortality to established plants. These changes in community composition can be viewed as secondary effects resulting from air pollution influencing plant competitive relationships (Bennett and Runeckles, 1977). The importance of seed factors and responses in maintaining community diversity and stability were recognized by early grassland ecologists (Davies, 1927; Blake, 1935; McAlister, 1943). That seed parameters such as weight and germination capacity can be altered by air pollutants has been demonstrated by several workers (Houston and Dochinger, 1977; Kohut *et al.*, 1978).

This portion of the ZAPS work concerns the development of methods and the assessment of sulfur dioxide influence on the seed characteristics of four major Northern Great Plains grassland species, *Poa sandbergii* (native bluegrass), *Koeleria cristata* (prairie junegrass), *Stipa viridula* (green needlegrass), and *Tragopogon dubius* (salsify). The major parameters examined were:

Germination Capacity--the percentage of "experimental seeds" exhibiting 2 mm or more of root or shoot growth under growth chamber conditions adjudged suitable for that species.

Seedfill--the percentage of "experimental seeds" which contain an endosperm.

Germination Capacity of Filled Seeds--the percentage of filled seeds exhibiting successful (>2 mm) root or shoot emergence.

Weight per Seed--average weight per "experimental seed" equilibrated to ambient laboratory conditions.

Coefficient of Rate of Germination (CRG)--an index of how rapidly seeds that exhibit successful germination did germinate.

The CRG was adopted from Maguire (1962) and Young and Evans (1977). It can be expressed as:

$$\text{CRG} = \sum_{i}^{n} \frac{g_n - g_{(n-1)}}{n}$$

where: g_n = accumulated germination percentage on a given day

$g_{(n-1)}$ = accumulated germination percentage on the previous day

n = number of days incubation

The larger the CRG, the faster the germination response. Additionally, it is of interest to examine certain characteristics of the germination response curve as a function of time. These include the number of days to 50% total germination and the day of peak germination. Earlier work (Rice *et al.*, 1979) suggested that these various seed responses were potentially influenced by sulfur dioxide treatment.

MATERIALS AND METHODS

In 1977, seed heads were clipped from a minimum of ten subplots per treatment plot and combined to form a composite for the respective plots. The material was stored for one year to allow after-ripening. Seeds were then hand-stripped from the heads. Replicates (ten per plot) were obtained by apportioning the samples by weight. This is done to standardize the total tissue mass in the trial, and it is intended to reduce within sample variance and bias arising from visual selection of seed if direct counts are made from a limited total sample. When the total weight of seeds from any treatment plot was limited, the available material was apportioned among ten replicates. When larger seed volumes were available, enough seeds to obtain a standard weight was randomly selected from the sample. In both cases, the actual counted number of "experimental seeds" for each replicate was determined after completion of the incubation period.

Germination tests were continued for 30- or 45-day periods, depending on the species. Fungal and bacterial growth were restricted by dry dusting seeds with 50% Ortho Captan and using an Ortho solution (two teaspoons per liter) as the water supply for germination. The petri dishes and cellulose pads which supported the seeds were autoclaved (15 pounds-15 minutes). Growth chamber conditions are described in an earlier report (Rice *et al.*, 1979). Replicate positions in the chambers were randomized daily after the germination counts were made. The number of seeds germinating each day was recorded, and at the end of the trial period the number of ungerminated seeds was determined. Ungerminated seeds were tabulated as those containing an endosperm and those with an empty palea. Seeds which germinated or did not germinate but contained an endosperm were considered "filled seeds."

Growth chamber size and the daily counting task precluded running all ten replicates at the same time. A randomized complete block design was used to remove the contribution to error mean square resulting from staggering the replicates within the trial. Dunnett's (1955) procedure was used to compare the Off Plot Control 1 (OPC 1), Fifteen Mile Creek Control (15 Mi), and site I or site II treatment plots with the respective site I or II A plots. The daily germination counts were also used to compute the relative and cumulative relative frequency distributions of germination as a function of time. These curves were used to estimate the number of days for 50% germination and the day of peak germination. These two responses were examined by Friedman's (1937) nonparametric two-way analysis of variance. If the Friedman statistic indicated significance ($p \leq .05$), the plot with the most extreme rank sum was removed from the data set and another Friedman's test conducted until the set was nonsignificant. Conover (1971) cautions that the α calculated with repeated application of Friedman's test is not exact.

RESULTS

The error term for seed weight and each major response parameter determined in the germination trials is presented in Table 11.1. The comparisons of the various treatment plots with the respective A plots are portrayed in Tables 11.2 to 11.5 for each of the four species.

All five response variables for *Poa sandbergii* (Table 11.2) from ZAPS IA were lower than those from OPC 1. ZAPS IA germination responses were also lower than the ZAPS IB, IC and ID treatment. Seed weight between site I plots and ZAPS IA did not vary significantly. At site II, Germination Capacity, Seedfill, and Germination Capacity of Filled Seeds were lower on ZAPS IIB and IID than on the ZAPS IIA plot. Weight per Seed was also significantly lower on the ZAPS IIB and IID plots.

All four germination responses in *Koeleria cristata* from ZAPS IA were depressed relative to the OPC 1. This pattern of a depressed ZAPS IA plot is similar to that observed in *P. sandbergii*; however, the *K. cristata* experimental seeds were heavier on ZAPS IA, with weight declining on the higher

TABLE 11.1. 1977 SEED RESPONSE ERROR MEAN SQUARES*

	df ν_1,ν_2		Coefficient of Rate of Germination	Germination Capacity†	Seedfill†	Germination Capacity of Filled Seed†	Weight per Seed (mg)
Poa sandbergii	8,70	Error Mean Square	1.377	3.481	3.964	57.117	5.00×10^{-4}
Koeleria cristata	8,72	Error Mean Square	0.734	3.212	3.501	25.695	6.63×10^{-5}
Stipa viridula	5,42	Error Mean Square	3.304	10.534	N.D.‡	N.D.	2.64×10^{-2}
Tragopogon dubius	8,68	Error Mean Square	0.965	6.617	N.D.	N.D.	2.69×10^{-2}

*Derived from a randomized complete block design. All treatment effects measured were highly significant ($p < .001$).

†Values expressed as angular (arcsin) transform of percentage.

‡N.D. = Not Determined.

TABLE 11.2. *POA SANDBERGII* SEED RESPONSES COMPARED TO THE A PLOT† AT EACH SITE

Response	OPC 1	ZAPS IA	ZAPS IB	ZAPS IC	ZAPS ID
Coefficient of Rate of Germination	9.468*	8.073	8.551	10.485**	10.683**
Germination Capacity†	20.8 **	5.2	7.8 *	19.1 **	18.3 **
Seedfill‡	24.1 **	9.0	11.4 *	23.2 **	22.0 **
Germination Capacity of Filled Seeds‡	61.1_9 **§	34.3	44.8 *	56.3 **	57.6 **
Weight per Seed (mg)	0.265*	0.236	0.225	0.255	0.247
	OPC 1	**ZAPS IIA**	**ZAPS IIB**	**ZAPS IIC**	**ZAPS IID**
Coefficient of Rate of Germination	9.468	10.421	9.234_9 §	10.530	10.646
Germination Capacity‡	20.8	20.0	6.1_9 **§	18.5	8.5 **
Seefill‡	24.1	22.9	8.5 **	21.5	12.5 **
Germination Capacity of Filled Seeds‡	61.1	61.5	47.9_9 **§	60.2	43.9 **
Weight per Seed (mg)	0.265	0.251	0.204**	0.233	0.192**

*Significant, $p \leq .05$.

**Highly significant, $p \leq .01$.

†Dunnett's procedure for comparing several treatments to a "control."

‡Values are expressed as the angular (arcsin) transform of the percentage.

§Ten items per sample, except where subscript indicates otherwise ($\overline{X}_9$).

TABLE 11.3. *KOELERIA CRISTATA* SEED RESPONSES COMPARED TO THE A PLOT† AT EACH SITE

Response	OPC 1	ZAPS IA	ZAPS IB	ZAPS IC	ZAPS ID
Coefficient of Rate of Germination	15.145**	13.904	14.177	14.052	14.816
Germination Capacity‡	19.5 **	11.0	8.1 **	12.5	22.5 **
Seedfill‡	20.8 **	12.8	10.8	14.0	23.8 **
Germination Capacity of Filled Seeds‡	70.2 **	61.0	49.6 **	64.1	71.4 **
Weight per Seed (mg)	0.133**	0.161	0.144**	0.124**	0.146**
	OPC 1	ZAPS IIA	ZAPS IIB	ZAPS IIC	ZAPS IID
Coefficient of Rate of Germination	15.145	16.212	15.927	15.993	16.180
Germination Capacity‡	19.5	29.3	23.0	32.3	33.5
Seedfill‡	20.8	30.5	24.5	33.2	34.8
Germination Capacity of Filled Seeds‡	70.2	75.0	70.6	77.9	75.4
Weight per Seed (mg)	0.133*	0.151	0.150	0.153	0.176**

*Significant, $p \leq .05$.

**Highly significant, $p \leq .01$.

†Dunnett's procedure for comparing several treatments to a "control."

‡Values are expressed as the angular (arcsin) transform of the percentage.

TABLE 11.4. *STIPA VIRIDULA* SEED RESPONSES COMPARED TO THE A PLOT† AT EACH SITE

Response	15 Mi	OPC 1	ZAPS IIA	ZAPS IIB	ZAPS IIC	ZAPS IID
Coefficient of Rate of Germination	13.892	13.179	11.868	11.574	10.669_9§	8.876_9**§
Germination Capacity‡	14.7	15.1	17.3	17.4	10.1 **	9.1 **
Weight per Seed (mg)	2.801	2.477*	2.681	2.885*	2.875 *	2.550

*Significant, $p \leq .05$.

**Highly significant, $p \leq .01$.

†Dunnett's procedure for comparing several treatments to a "control."

‡Values are expressed as the angular (arcsin) transform of the percentage.

§Ten items per sample, except where subscript indicates otherwise ($\bar{X}_9$).

TABLE 11.5. *TRAGOPOGON DUBIUS* SEED RESPONSES COMPARED TO THE A PLOT† AT EACH SITE

Response	15 Mi	ZAPS IA	ZAPS IB	ZAPS IC	ZAPS ID
Coefficient of Rate of Germination	31.735_{9}*§	30.374_{9}§	30.480	30.318	29.903
Germination Capacity‡	80.9 **	86.8	82.6 **	84.6	81.8 **
Weight per Seed (mg)	6.74 **	6.14_{9}§	5.52_{9}**§	5.62 **	5.38 **
	15 Mi	ZAPS IIA	ZAPS IIB	ZAPS IIC	ZAPS IID
Coefficient of Rate of Germination	31.735_{9}§	32.589_{9}§	32.670	31.696	31.309*
Germination Capacity‡	80.9 **	86.2	83.2 *	83.4	86.0
Weight per Seed (mg)	6.74 *	6.32	6.19	6.00 **	5.98 *

*Significant, $p \leq .05$.

**Highly significant, $p \leq .01$.

†Dunnett's procedure for comparing several treatments to a "control."

‡Values are expressed as the angular (arcsin) transform of the percentage.

§Ten items per sample, except where subscript indicates otherwise ($\bar{X}_9$).

treatment levels on site I. Germination Capacity and Germination Capacity of Filled Seeds were depressed on ZAPS IB but elevated relative to ZAPS IA on the ID plot. Seedfill was also higher on ZAPS ID. Germination parameters were similar on all plots at site II. The low *K. cristata* seed weight found in the OPC 1 sample was still significant compared with the site IIA plot. Additionally, the heaviest experimental seed was derived from the ZAPS IID plot sample.

Stipa viridula was collected from site II and two plots (OPC 1 and 15 Mi) located away from either of the fumigation sites. The Coefficient of Rate of Germination was lowest for seeds from ZAPS IID. Germination Capacity was depressed for both the ZAPS IIC and IID seeds. Seeds from OPC 1 were lightest, while those from ZAPS IIB and IIC were heavier than the site II control.

Tragopogon dubius from 15 Mi germinated faster and was heavier than that from the ZAPS IA plot (Table 11.5). However, the Germination Capacity was slightly higher (99.7% vs. 97.5%, back transform of the arcsin) for the ZAPS IA sample. In seeds from ZAPS IB and ID, Germination Capacity was reduced, while seed weight was lower on site I plots relative to the control. The slight depression in Germination Capacity observed for 15 Mi *T. dubius* seed was also significant relative to the ZAPS IIA plot (97.5% on 15 Mi vs. 99.6% on ZAPS IIA back transform). Seed weight remains highest for the 15 Mi seeds. Weight per Seed and the Coefficient of Rate of Germination were depressed on ZAPS IID relative to ZAPS IIA. Seed weight was also depressed on ZAPS IIC and germination capacity on ZAPS IIB.

The relative frequency distributions of total germination success for each day of incubation are portrayed graphically for each species by treatment plot in Figures 11.1 through 11.4. These graphs are formed from the pooled data for all replicate trials. The day of peak germination can be seen on these histograms and days to 50% germination is approximately indicated by the arrow. Day of peak germination of the individual replicates was analyzed by Friedman's test (Table 11.6). *T. dubius* was not treated in this analysis, as 86 of the 90 cases peaked on day three. The only significant difference detected was the high rank sum representing the delayed peak day for the *Poa sandbergii* sample from ZAPS IIB. The pooled responses for this sample suggest a bimodality (Figure 11.1) which may have led to an overestimate of peak for this species.

The relative cumulative frequency distributions as a function of the natural log of the number of days of incubation are portrayed in Figures 11.5 through 11.8. These graphs are also derived from the pooled data for each species and treatment plot. The time required for germination of 50% of the seeds was estimated by linear interpolation of the counts for the day preceeding and following this event for each individual replicate. This variable was analyzed by Friedman's test and the results are indicated in Table 11.7. Comparisons for a species are made within a site and include either OPC 1 or 15 Mi when the sample was available. The higher rank sums within a column indicate longer time periods for 50% germination response. For *Poa sandbergii* at site I, the ZAPS IA and IB samples were delayed in reaching 50% germination

TABLE 11.6. DAY OF PEAK GERMINATION†

Plot	Rank Sum	Mean	Plot	Rank Sum	Mean
Poa sandbergii					
OPC 1	31.5	9.4	OPC 1	31.5	9.4
ZAPS IA	38.5	11.4	ZAPS IIA	24.0	8.6
ZAPS IB	32.5	9.9	ZAPS IIB	43.0	10.6*
ZAPS IC	23.0	8.8	ZAPS IIC	28.5	8.8
ZAPS ID	24.5	8.5	ZAPS IID	23.0	9.4
Koeleria cristata					
OPC 1	21.0	5.8	OPC 1	32.0	5.8
ZAPS IA	29.0	6.3	ZAPS IIA	27.0	5.6
ZAPS IB	31.5	6.4	ZAPS IIB	29.5	5.7
ZAPS IC	36.0	6.4	ZAPS IIC	27.0	5.6
ZAPS ID	32.5	6.3	ZAPS IID	34.5	5.9
Stipa viridula					
			15 Mi	25.5	5.7
			OPC 1	29.0	6.7
			ZAPS IIA	38.0	9.2
			ZAPS IIB	42.5	12.0
			ZAPS IIC	43.5	8.1
			ZAPS IID	31.5	10.1

*Significant, $p \leq .05$.

†Friedman's test.

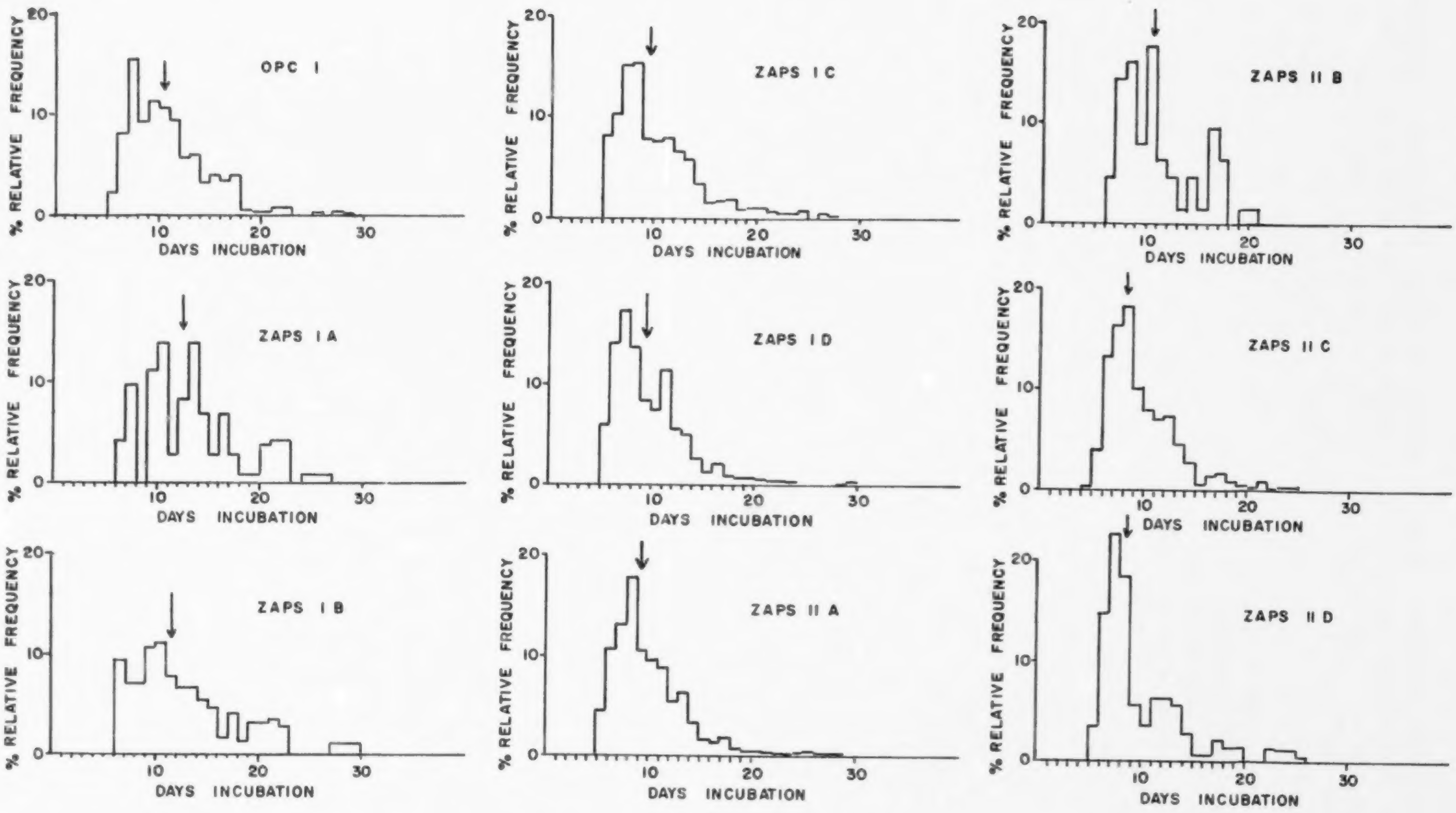

Figure 11.1. Histogram of successfully germinating *Poa sandbergii* seeds on OPC 1 and ZAPS I and II. ↓ indicates days to 50% germination.

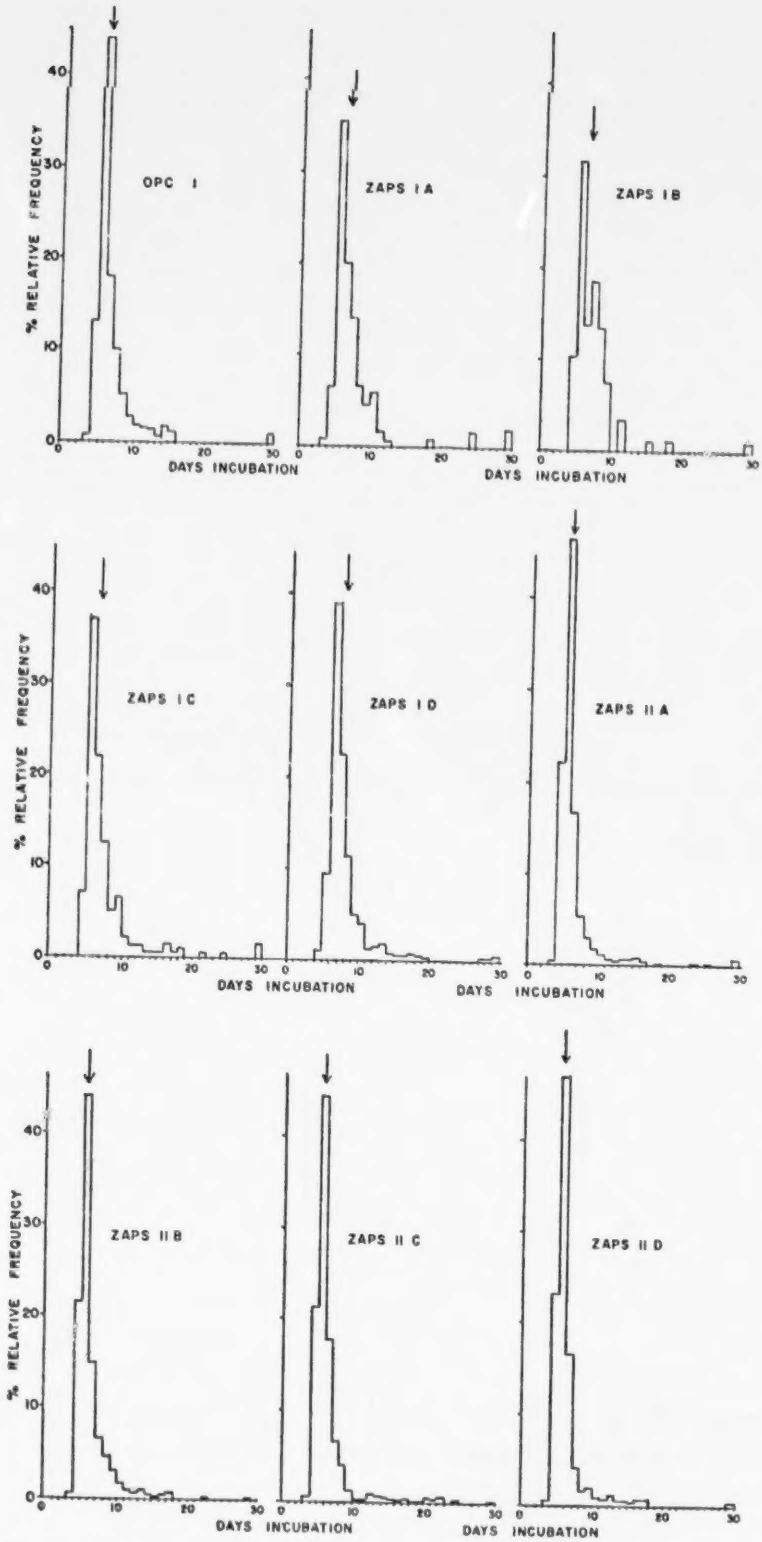

Figure 11.2. Histogram of successfully germinating *Koeleria cristata* seeds on OPC 1 and ZAPS I and II. ↓ indicates days to 50% germination.

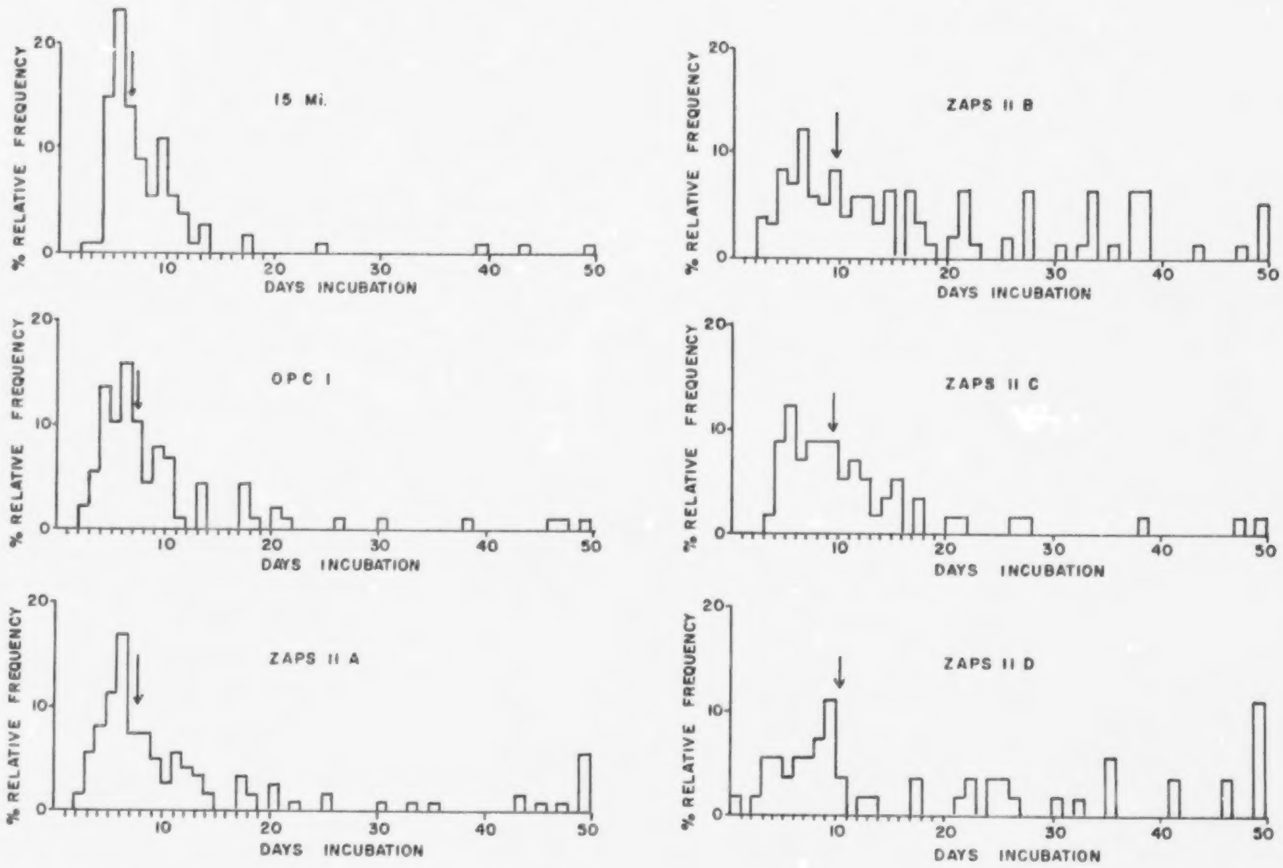

Figure 11.3. Histogram of successfully germinating *Stipa viridula* seeds on 15 Mi, OPC 1 and ZAPS II. ↓ indicates days to 50% germination.

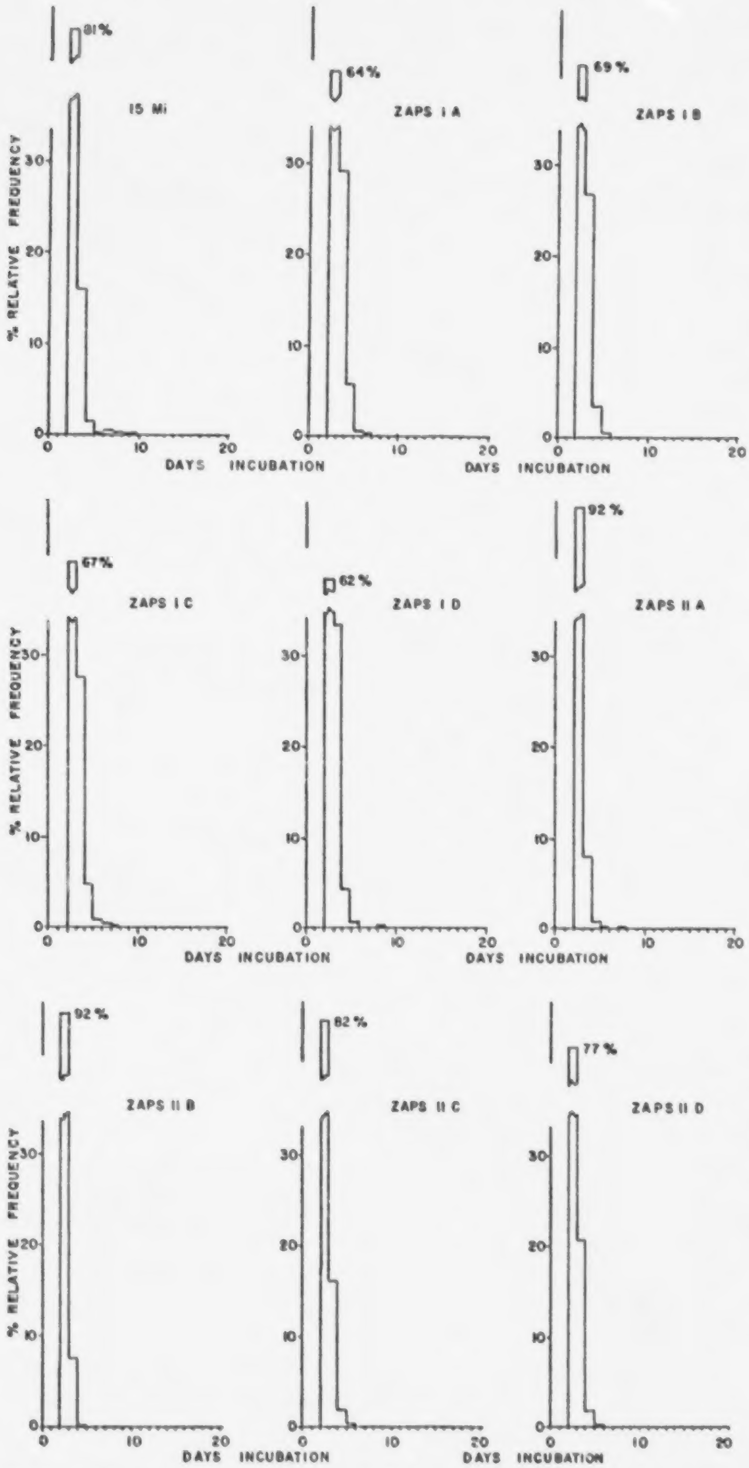

Figure 11.4. Histogram of successfully germinating *Tragopogon dubius* seeds on 15 Mi and ZAPS I and II.

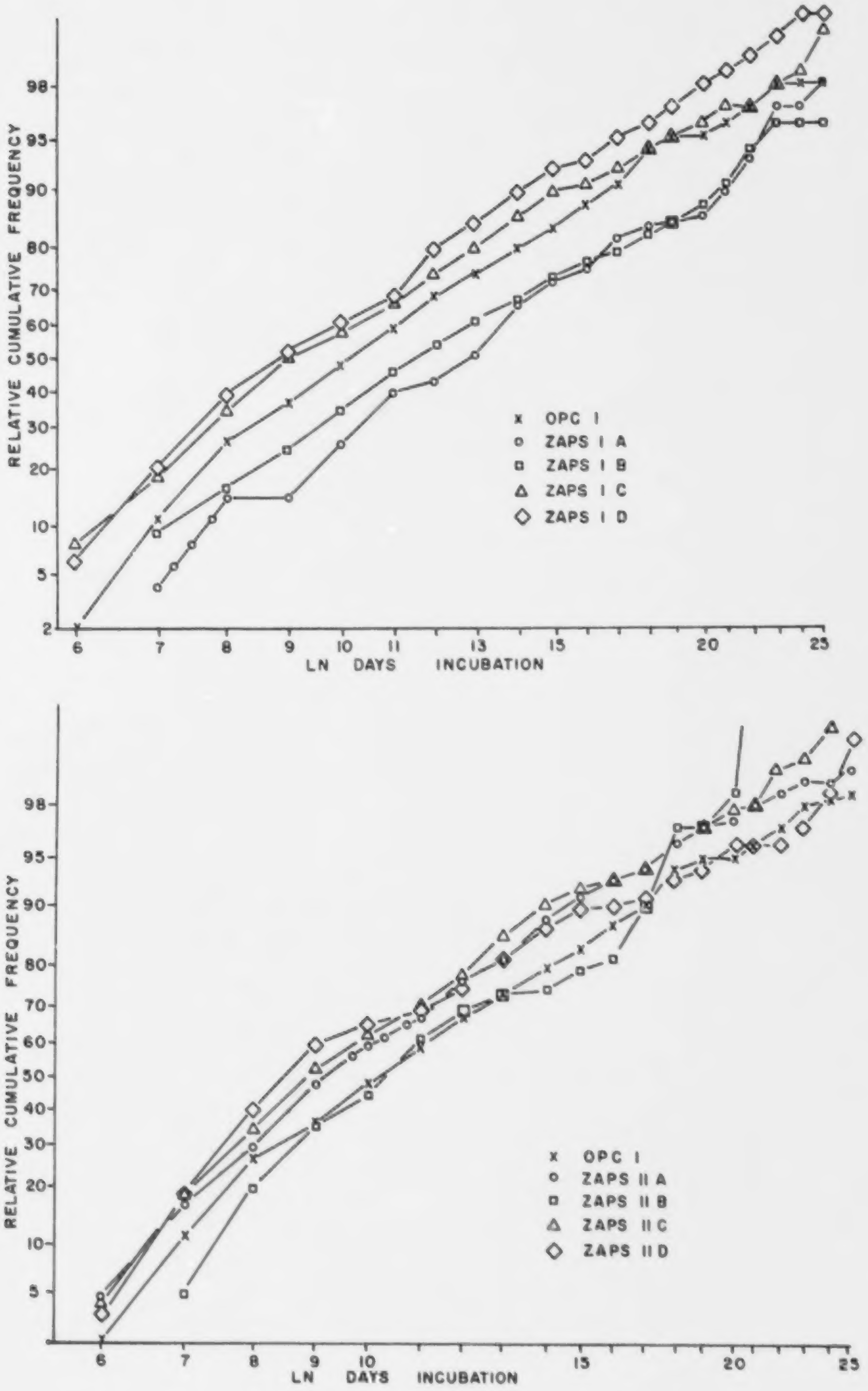

Figure 11.5. Cumulative germination of *Poa sandbergii* seeds from ZAPS I and II as a function of time.

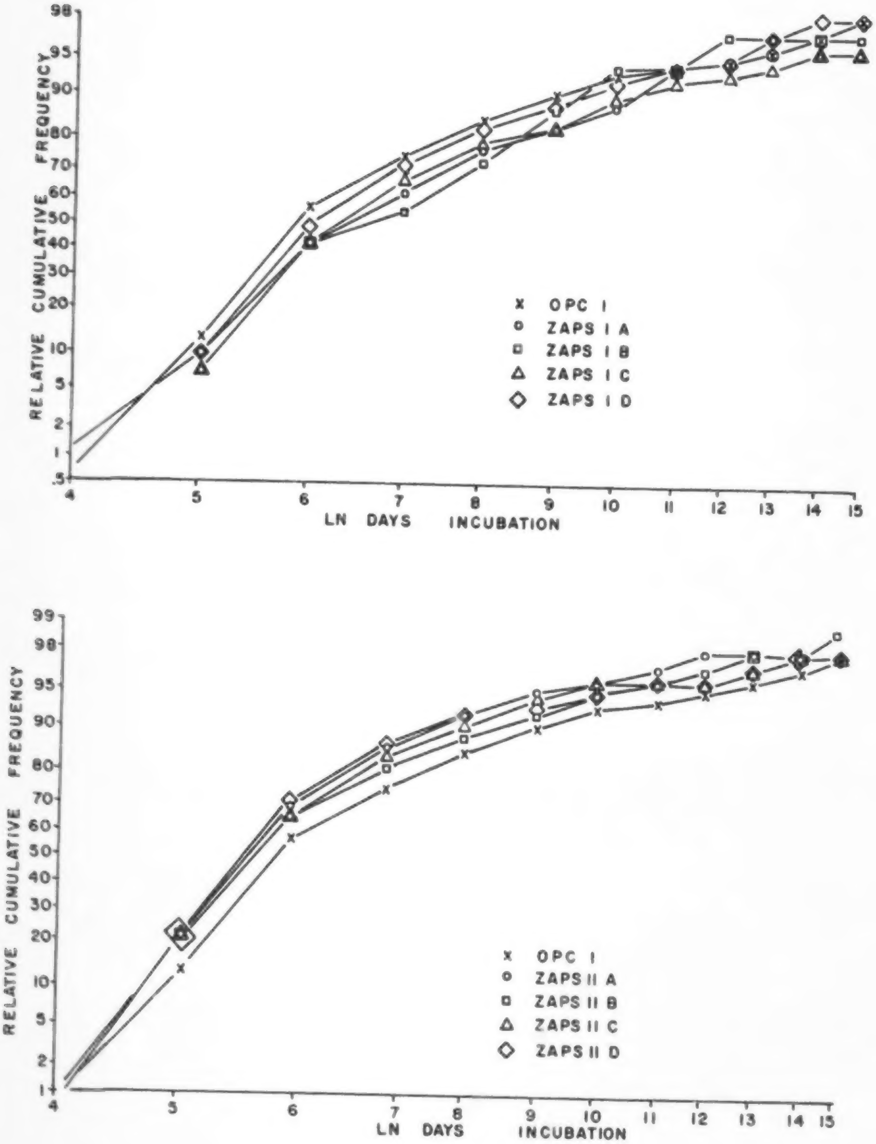

Figure 11.6. Cumulative germination of *Koeleria cristata* seeds from ZAPS I and II as a function of time.

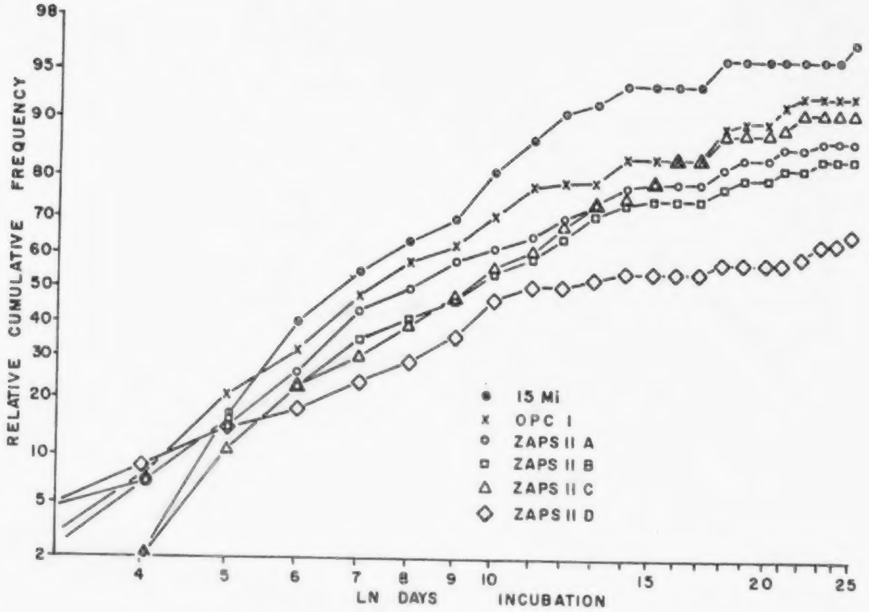

Figure 11.7. Cumulative germination of *Stipa viridula* seeds from ZAPS II as a function of time.

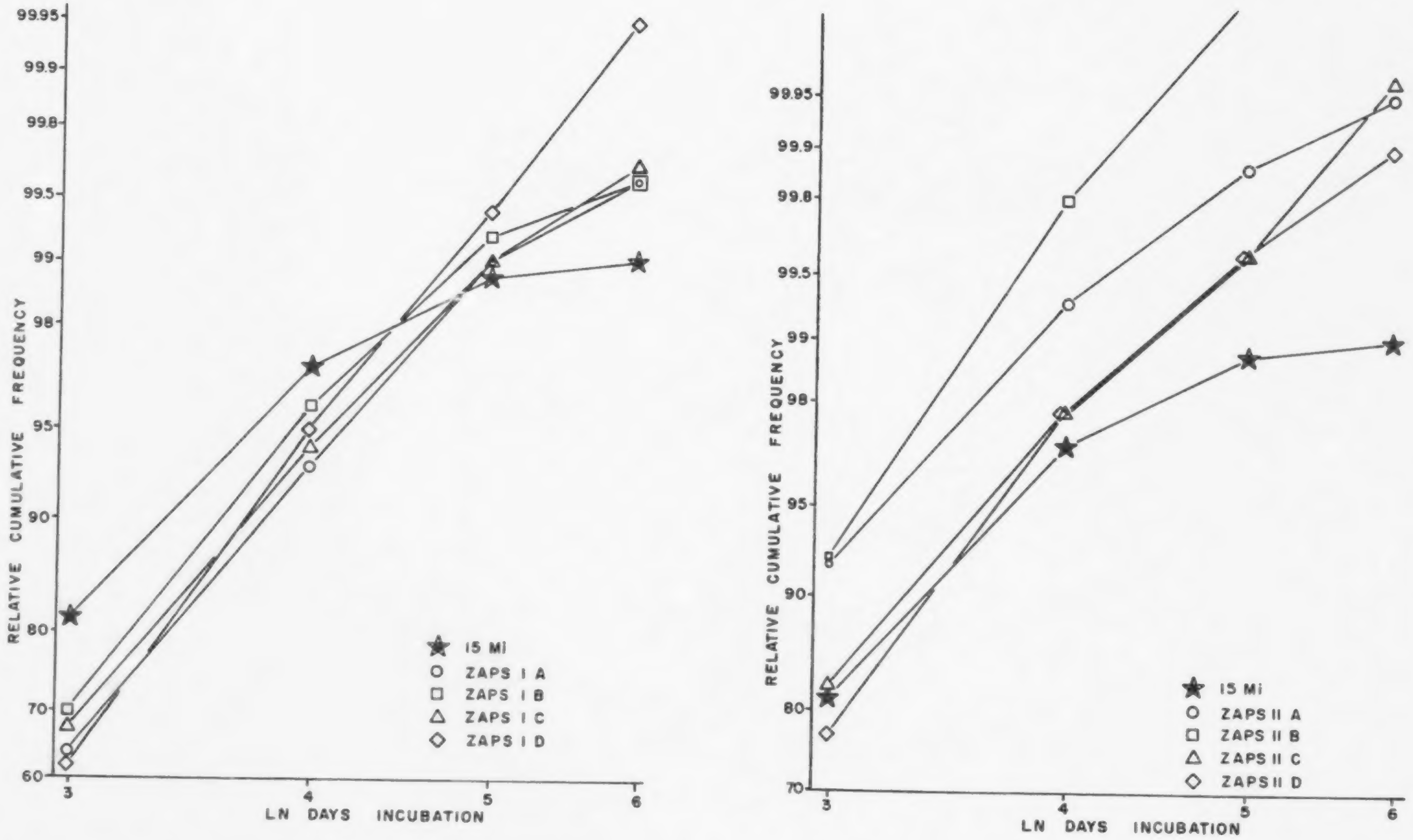

Figure 11.8. Cumulative germination of *Tragopogon dubius* seeds from ZAPS I and II as a function of time.

response. In the site II contrast, the OPC 1 sample was slower than the site II sample. *Koeleria cristata* from site I exhibited an earlier 50% response in the OPC 1 and ZAPS ID samples. Site II seeds were faster than the OPC 1 seeds but exhibited no difference between site II samples themselves. *Stipa viridula* reached 50% germination most rapidly in seeds from 15 Mi and OPC 1, while ZAPS IID was significantly slower. *Tragopogon dubius* seeds from OPC 1 reached 50% germination before any of the site I seeds. From site II, the ZAPS IIA and IIB seeds were half done before seeds from any of the other treatments.

TABLE 11.7. DAYS TO 50% GERMINATION†

Plot	Rank Sum	Mean	Plot	Rank Sum	Mean
Poa sandbergii					
OPC 1	29.5	10.16	OPC 1	39.0	10.16*
ZAPS IA	40.5	12.25*	ZAPS IIA	24.5	9.03
ZAPS IB	38.0	11.48*	ZAPS IIB	37.0	10.32
ZAPS IC	20.0	9.48	ZAPS IIC	28.5	9.12
ZAPS ID	22.0	8.87	ZAPS IID	21.0	8.76
Koeleria cristata					
OPC 1	18.0	5.85*	OPC 1	44.5	5.85*
ZAPS IA	38.0	6.60	ZAPS IIA	23.5	5.50
ZAPS IB	34.0	6.41	ZAPS IIB	24.5	5.52
ZAPS IC	39.0	6.27	ZAPS IIC	30.5	5.55
ZAPS ID	21.0	5.92*	ZAPS IID	27.0	5.55
Tragopogon dubius					
15 Mi	18.5	2.62*	15 Mi	38.5	2.62
ZAPS IA	35.0	2.82	ZAPS IIA	17.5	2.55*
ZAPS IB	28.0	2.74	ZAPS IIB	17.5	2.55*
ZAPS IC	29.5	2.81	ZAPS IIC	37.5	2.62
ZAPS ID	39.0	2.84	ZAPS IID	39.0	2.66
Stipa viridula					
			15 Mi	17.5	6.69*
			OPC 1	23.0	7.21*
			ZAPS IIA	35.5	8.53
			ZAPS IIB	40.5	9.18
			ZAPS IIC	39.5	8.75
			ZAPS IID	54.0	16.48*

*Significant, $p \leq .05$.

†Friedman's test.

DISCUSSION

Seed parameters for all significant responses except seed weight in *K. cristata* and a slight difference in germination capacity for *T. dubius* demonstrate that seed viability and vigor on the ZAPS IA "control" is depressed relative to the control plots (OPC 1 and 15 Mi) removed from the ZAPS I site. Increases in germination response parameters for *P. sandbergii* and *K. cristata* at ZAPS IB, IC and ID thus may well reflect a relative depression in vigor of plants on the ZAPS IA plot. We concluded that edaphic conditions at ZAPS IA are relatively harsh, and this is also reflected in the apparent difference in community structure as evident by the presence of *Festuca idahoensis* on the plot and the proximity of the *Artemisia tridentata* stands. This discontinuity may extend into the ZAPS IB treatment plot.

The weedy biennial, *T. dubius*, responded with reduced germination capacity as the sulfur dioxide level increased on site I. Although this reduction in Germination Capacity was significant, the magnitude of the back transformation difference (<2%) is quite small compared to total germination success which ranges from 97% to 99%, and shifts of this relative magnitude may be too minor to have much effect on seedling competition between species.

At site II, Germination Capacity, Seedfill, and Germination Capacity for *P. sandbergii* were reduced at ZAPS IIB and IID levels. The ZAPS IIC plot was similar to IIA. This relative vitality of *P. sandbergii* seed produced on the ZAPS IIC plot was also evident in the 1976 trials (Rice *et al.*, 1979) and appears to be sufficient to overwhelm any treatment effect. For *K. cristata* at site II, germination responses were unaffected. The reduction in Germination Capacity for *S. viridula* on site II plots C and D parallels a similar but nonsignificant trend observed in 1976. There was decreased Germination Capacity response with increasing treatment level and a reduction in the Coefficient of Rate of Germination on ZAPS IID. The 50% germination point was delayed for *S. viridula* from ZAPS IID. Germination success for *S. viridula* from all plots (untransformed % = 6.4) remains an order of magnitude below that possible (83%) (Eddleman, 1977) for this species under the germination conditions employed. The characteristic dormancy reported of this species (McAlister, 1943; Rogler, 1960) is not being broken by the procedures currently employed. Cold water stratification will be employed on the 1978 collections to bring the total germination closer to its potential. For *T. dubius* at site II, the Coefficient of Rate of Germination tends to decline with increasing sulfur dioxide treatment, the decline being significant on the ZAPS IID plot, 50% of the germination response being reached sooner on ZAPS IIA and IIB than IIC and IID. The CRG's are so high (30+) for *T. dubius* compared to the perennial grasses (8 to 16) that it is unlikely the small decrease on the ZAPS IID plot would alone alter the competitive relationship between this weed and the grasses.

If the sulfur dioxide treatments are stressing the entire plant, it is reasonable to assume that available photosynthate would be prioritized for maintaining the vegetative top growth. Depressed fruit and/or seed yields for gas-stressed plants are frequently reported (Houston and Dochinger, 1977; Pack and Sulzback, 1976; Oshima *et al.*, 1977). Average seed weights were contrasted to their respective site I or II controls (A plots) for 21 cases, each case being a specific combination of species and intentional sulfur dioxide fumigation level (B, C or D plot treatment). No difference could be discerned in 8 cases, 3 cases suggested heavier seeds, while 10 cases indicated a significant ($p \leq .05$) decline in seed weight with sulfur dioxide treatment.

It was not determined what proportion of the depressed seed weights are a reflection of smaller seeds or due to lack of fill or seed set. Seed weight was derived from the sample weight divided by the total number of experimental seeds. This included both filled and unfilled "seed." Among the perennial grass seed collection for 1977, the average weight from each of the plots is less than the lower range of seed weights for the 1976 collections (Rice *et al.*, 1979). For the biennial *T. dubius*, the 1977 average weight range (5.38 to 6.76 mg/seed) overlaps the lower values for the 1976 weight range (6.04 to 7.58 mg/seed). Two factors potentially contribute to this general decline in seed weight. First, available soil moisture and precipitation was lower (Dodd *et al.*, 1979) during the seed set and development period (May, June, July) in 1977 than 1976. The moisture conditions for this period in 1977 were below the long-term averages. Second, the differences in seed head cleaning procedures may have increased the proportion of unfilled seed in the total experimental seed for the 1977 trials. Cleaning procedures for the 1978-79 collections will be more closely supervised and the experimental procedure altered to determine seed fill and seed weights prior to the actual germination trial.

CONCLUSIONS

The ZAPS IA plot appears to provide a poor habitat for seed production. This makes interpretation of site I seed responses difficult in terms of the single factor effect of sulfur dioxide treatment. A form of multi-variate analysis would be of value here but is beyond the sampling resources of the experimenter. Site II provides evidence of the depressing effect of sulfur dioxide on germination in three of the four species studied. Seed weights generally decline with increasing fumigation levels at both site I and II. Although responses are generally depressed with increasing gas treatment, the data does not suggest a response linear to the treatment gradient. Thus, the depressions may reflect edaphic factors or reflect inherent differences in the plant population across the sites. Procedural progress was made with the 1977 trials and will be adapted for testing the 1978-79 seed collections.

REFERENCES

Bennett, J. P., and V. C. Runeckles. 1977. Effects of Low Levels of Ozone on Plant Competition. J. Appl. Ecol., 14(3):877-880.

Blake, A. K. 1935. Viability and Germination of Seeds and Early Life History of Prairie Plants. Ecol. Monog., 5:407-460.

Conover, W. J. 1971. Practical Nonparametric Statistics. John Wiley and Sons, Inc., New York. 462 pp.

Davies. W. 1927. Seed Mixture Problems: Soil Germination, Seedling and Plant Establishment with Particular Reference to the Effects of Environmental and Agronomic Factors II. Field Trials. Welsh Plant Breeding Station. Bul. Series H. No. 6. pp. 39-60.

Dodd, J. L., W. K. Lauenroth, R. G. Woodmansee, G. L. Thor, and J. Chilgren. 1979. Soil, Chemical, and Meteorological Characteristics at ZAPS. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 331-376.

Dunnett, C. W. 1955. A Multiple Comparison Procedure for Comparing Several Treatments with a Control. J. Am. Stat. Assoc., 50:1096-1121.

Eddleman, L. E. 1977. Indigenous Plants of Southeastern Montana. I. Viability and Suitability for Reclamation in the Fort Union Basin. Special Publication Four. Montana Forest and Conservation Experiment Station, School of Forestry, University of Montana, Missoula, Mt. 122 pp.

Farrar, J. F., J. Ralton, and A. J. Rutter. 1977. Sulphur Dioxide and the Scarcity of *Pinus sylvestris* in the Industrial Pennines. Environ. Pollut., 14: 63-68.

Friedman, M. 1937. The Use of Ranks to Avoid the Assumption of Normality Implicit in the Analysis of Variance. J. Amer. Statis. Assoc., 32:675-701.

Houston, D. B., and L. S. Dochinger. 1977. Effects of Ambient Air Pollution on Cone, Seed and Pollen Characteristics in Eastern White and Red Pines. Environ. Pollut., 12:1-5.

Kohut, R. J., S. V. Krupa, and F. Russo. 1978. An Open-Top Chamber Study to Evaluate the Effects of Air Pollution on Soybean Yield. In: Proc. of 4th Joint Conference on Sensing of Environmental Pollutants, American Chemical Society, Washington, D.C. pp. 71-73.

Maguire, J. D. 1962. Speed of Germination-Aid in Selection and Evaluation for Seedling Emergence and Vigor. Crop Science, 2:176-177.

McAlister, D. F. 1943. The Effect of Maturity on the Viability and Longevity of the Seeds of Western Range and Pasture Grasses. J. Amer. Soc. Agron., 35:442-453.

Oshima, R. J., P. K. Braegelmann, D. W. Baldwin, V. Van Way, and O. C. Taylor. 1977. Reduction of Tomato Fruit Size and Yield by Ozone. J. Amer. Soc. Hort. Sci., 102: 289- 293.

Pack, M. R., and C. W. Sulzback. 1976. Response of Plant Fruiting to Hydrogen Fluoride Fumigation. Atmos. Environ., 10:73-81.

Rice, P. M., L. H. Pye, R. Boldi, J. O'Loughlin, P. C. Tourangeau, and C. C. Gordon. 1979. The Effects of "Low Level SO_2" Exposure on Sulfur Accumulation and Various Plant Life Responses of Some Major Grassland Species on the ZAPS Sites. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 494-591.

Rogler, G. A. 1960. Relation of Seed Dormancy of Green Needlegrass (*Stipa viridula*) to Age and Treatment. Agron. J., 52:467-469.

Westwood, W. E. 1979. Oxidant Effects on Californian Coastal Sage Scrub. Science, 205: 1001-1003.

Winner, W. E., and J. D. Bewley. 1978. Contrasts Between Bryophyte and Vascular Plant Synecological Responses in an SO_2-Stressed White Spruce Association in Central Alberta. Oecologia (Berl.), 33:311-325.

Young, J. A., and R. A. Evans. 1977. Squirrel Tail Seed Germination. J. Range Manag., 30: 33-36.

SECTION 12

EFFECTS OF CONTROLLED SO_2 EXPOSURE ON NET PRIMARY PRODUCTION AND PLANT BIOMASS DYNAMICS

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ABSTRACT

Objectives of this study were to determine effects of low level SO_2 fumigation on above- and belowground plant biomass dynamics and aboveground net primary production. With one exception we were unable to measure significant effects on these parameters. Rates of increase in rhizome biomass over the four-year period of the study were reduced by SO_2 and indicate that long term exposure of this grassland to low level SO_2 will ultimately reduce the vigor of the perennating organs to a point where above- and belowground biomass dynamics will be altered, net primary production will be reduced, and species composition will be modified.

INTRODUCTION

Because of the increase in electrical power production in the Northern Great Plains, air pollution is expected to increase (Northern Great Plains Resource Program, 1975). Aside from the power industry itself, the main industry of the region is the range livestock industry. It is quite possible that the altered air quality will affect the livestock industry. As with other rangelands, these rangelands are native ecosystems that are complex combinations of indigenous plant and animal species that interact with each other and their physical environment in capturing, storing, and utilizing energy in a self-sustaining manner. Under normal circumstances, domestic livestock production can be fit into this process quite profitably without interfering with the self-sustaining character of the ecosystem processes (Lewis, 1969). Further, livestock production on rangelands is closely associated with the quantity and quality of aboveground forage production. The threat that air pollution poses to a livestock industry based on native rangeland is threefold: direct effect of air pollutants on the livestock, direct effect on quantity and quality of forage production, and effects on the self-sustaining properties of the ecosystem. The last two of these questions are considered for one grassland type in this study.

Several investigators have reported biomass dynamics and net primary production estimates for the northern mixed grass prairie under pollution-free conditions (Coupland, 1973; Lauenroth and Whitman, 1977; Lauenroth *et al.*, 1975; Dodd *et al.*, 1974). These studies indicate rapid increases of plant biomass in late May, June, and early July and aboveground net primary productivity estimates ranging from 100 to 200 $g \cdot m^{-2} \cdot yr^{-1}$. Although the species composition of these grasslands is dependent on specific site factors, such as soils, rainfall, and grazing history, these grasslands are nearly always dominated by cool season grass species, and in most cases *Agropyron smithii* Rydb. is the main species. Other cool season grass or grasslike species of importance throughout the region are *Stipa comata* Trin. & Rupr., *S. viridula* Trin., *Koeleria cristata* (L.) Pers., *Calamagrostis montanensis* Scribn., *Carex* spp., *Bromus* spp., and other species of *Agropyron*.

Although much research has been done on effects of air pollution on plants of ornamental or agronomic importance (see reviews by Daines, 1968; Mudd and Kozlowski, 1975; Treshow, 1970), comparatively little has been conducted on effects on productivity of the native plant species of the semi-arid rangelands of the western United States. Recent studies indicate that grasses of semi-arid rangelands are more resistant to air pollution than are agronomic species (Hill *et al.*, 1974; Bennet and Hill, 1973; Davis *et al.*, 1966; Tingey *et al.*, 1976). However, Bell and Clough (1973) have found significant reduction in yield of a cool season grass, *Lolium perenne*, exposed to low-level SO_2 (12 pphm) for several weeks.

The objectives of this study were to determine the short- and near-term effects of exposure to low-level SO_2 concentration on intraseasonal biomass dynamics and aboveground net primary productivity (ANPP) of a northern mixed grass prairie. We hypothesized that low-level exposures of SO_2 throughout the growing season would reduce ANPP and change species and species group contributions to total ANPP.

MATERIALS AND METHODS

Precipitation was measured on each study site between May and September in each year. Soil water content to a depth of 105 cm was determined gravimetrically on each treatment plot throughout each growing season. Sampling frequency varied from weekly to monthly during the study.

Aboveground plant biomass was sampled on six dates in 1975 and 1976, in mid-July in 1977 and in mid-May and mid-July in 1978. Quadrats were located randomly, and vegetation was clipped and separated by species into current live, recent dead (current year's dead) and old dead (previous year's dead). Samples were oven dried at 60°C to constant weight and weighed. In 1975, ten circular 0.5-m^2 quadrats were sampled in each treatment on each date. This was changed to 20 circular 0.25-m^2 quadrats in 1976, 1977, and 1978 to increase precision.

Although numerous computational procedures exist for estimating ANPP from harvest data (Singh *et al.*, 1975), only one procedure was appropriate for all treatments in all years of this study: the total standing crop of current production as measured in mid-July. The more accurate procedure (summation of species and species group peaks of current production from frequent harvest data) could be used only in 1975 and 1976, when we sampled monthly throughout the growing season. We computed ANPP estimates by both procedures for 1975 and 1976. The summation of peaks procedure resulted in an 18% (SE = 3%) greater estimate of ANPP than did the July standing crop estimate. A comparable difference between the two procedures was reported for a South Dakota grassland of similar botanical character (Dodd *et al.*, 1974). Therefore, our estimates of ANPP are July standing crop estimates increased by 18%.

Belowground plant biomass to a depth of 10 cm was sampled monthly (May-September) in 1975, 1976, and 1978 and in mid-July in 1977. On each sampling date two belowground samples were taken from each herbage sample location after harvest of the aboveground material. Samples were secured with a 7.5 cm diameter × 10 cm long steel cylinder.

Plant material was separated from soil by the washing-flotation procedure described by Lauenroth and Whitman (1971) and separated into roots, rhizomes, and crowns (basal parts of shoots that are subterranean but positioned above the transition zone of the plant). This washing-flotation procedure retains all live and dead belowground plant material that will not pass through a 60-mesh $\cdot$ in^{-1} screen. Separated materials were oven dried to a constant weight, ashed at 600°C, and reweighed; results are reported on an ash-free organic matter basis.

RESULTS AND DISCUSSION

Precipitation and Soil Water

Early growing season precipitation (May-July) was near normal in 1977, 40% greater than normal in 1975, and more than twice normal in 1978 (Table 12.1). Although early season precipitation in 1976 was nearly 40% above normal on Site I, it was only slightly above normal on Site II. August and September precipitation was low and near normal in all years. Soil water storage was high in early season and rapidly depleted in July and August in all years (Figure 12.1).

Aboveground Biomass Dynamics

In 1975 and 1976 seasonal biomass dynamics for the total plant community consisted of an early period of rapid increase (late April to early July), followed by a period of slight increase (1975) or a slight decrease (1976) in standing crop of current seasons production (Figure 12.2). The cessation of rapid growth was associated with diminished rainfall and rapid exhaustion of soil water (Figure 12.1). The SO_2 treatments did not appear to alter the timing or rates of standing crop increases during the rapid growth period, nor did the treatment alter the post-peak biomass dynamics.

Community biomass dynamics were largely a reflection of the biomass dynamics of western wheatgrass (Figure 12.3) and to a lesser extent of prairie Junegrass (Figure 12.4). Although the low frequency of standing crop estimates prohibits precise evaluation, western wheatgrass appeared to have its most

TABLE 12.1. GROWING SEASON PRECIPITATION* FOR SITES I AND II, 1975-1978, COMPARED WITH LONG-TERM AVERAGE (1932-1977) AT BROADUS, MONTANA†

Month	1975 Site	1976 Site		1977 Site		1978 Site		Long-term average
	I	I	II	I	II	I	II	
May	104	97	44	45	46	263	276	57
June	109	102	94	91	98	57	56	80
July	29	38	41	18	14	28	30	35
August	14	10	17	18	18	13	11	27
September	6	14	14	36	40	No data		30
May-July	242	237	179	154	158	348	362	172

* mm

† Broadus, Montana, is located 40 km northeast of the study sites.

rapid aboveground growth period later in the season than did prairie Junegrass. Also, western wheatgrass standing crop appeared to persist longer after the rapid growth period than did prairie Junegrass. Treatment-induced changes in biomass dynamics of these two species were not detected.

Belowground Biomass Dynamics

Belowground plant biomass dynamics were dominated by changes in root biomass. Even though root standing crops were estimated monthly during the growing season on all treatments in 1975, 1976, and 1978, we found consistent dynamics only in 1978 (Table 12.2). From May to July, root biomass decreased by about 25% and from July to September increased by about the same amount. This demonstration of a mid-season low is generally consistent with findings of other studies conducted in the northern mixed grass priarie (Lauenroth and Whitmann, 1977; Dodd *et al.*, 1974; Lewis *et al.*, 1971) and simulations of belowground dynamics (Bartos and Jameson, 1974; Detling *et al.*,1979).

The SO_2 treatments did not alter seasonal changes in root biomass in 1978. Because the 1975 and 1976 monthly estimates of root biomass were more erratic, we could not test the effects of SO_2 treatments on seasonal changes in root biomass in these years.

Total belowground plant biomass increased over the course of the experiment on the controls and all treatments on both sites (Table 12.3). On Site I

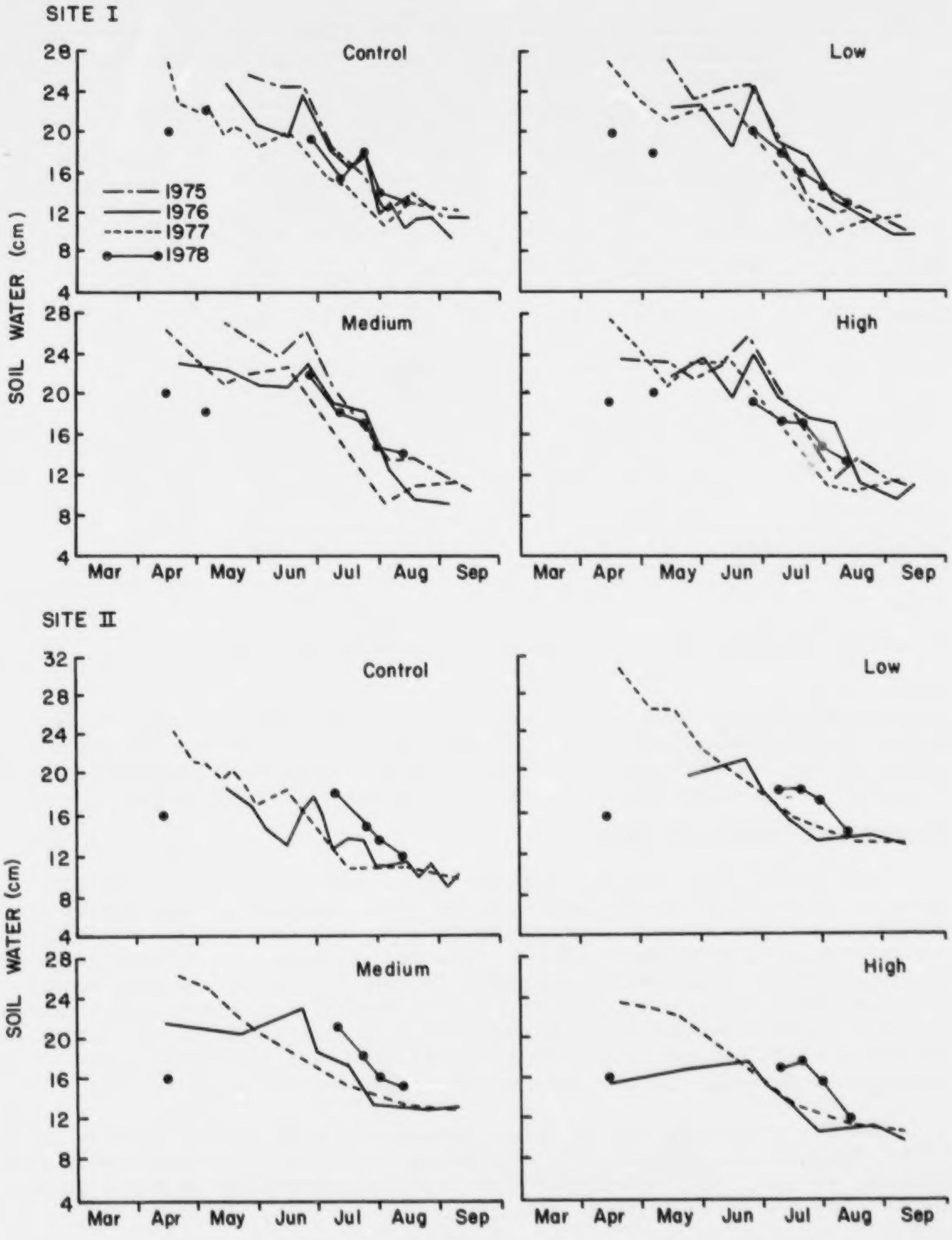

Figure 12.1. Soil water dynamics within 0-105 cm depth for Sites I and II, 1975-1978.

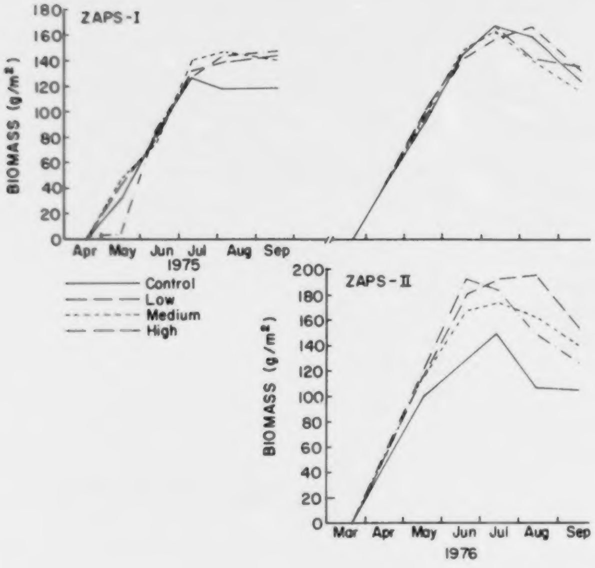

Figure 12.2. Seasonal change in current years production for all plant species combined, ZAPS I and II, 1975 and 1976.

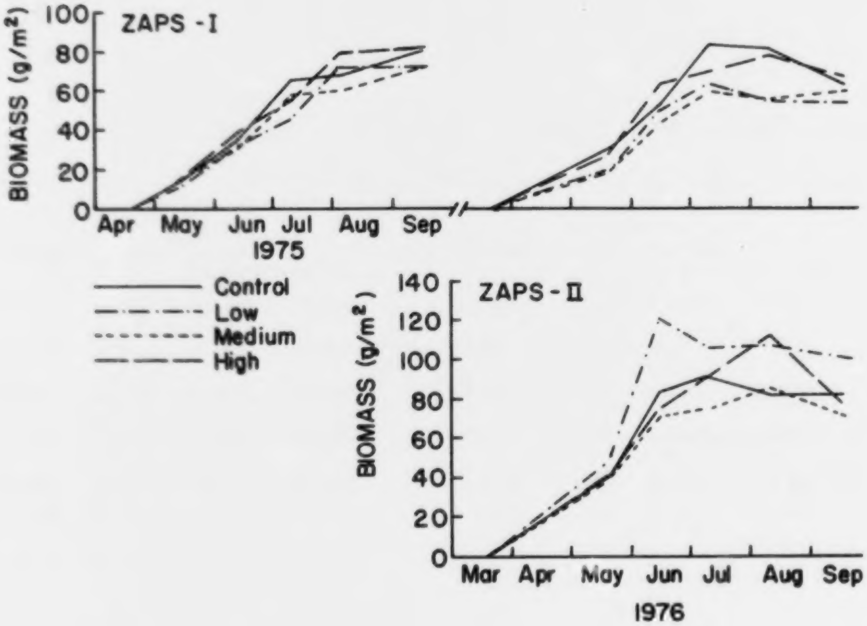

Figure 12.3. Seasonal change in current year's production for western wheatgrass, ZAPS I and II, 1975 and 1976.

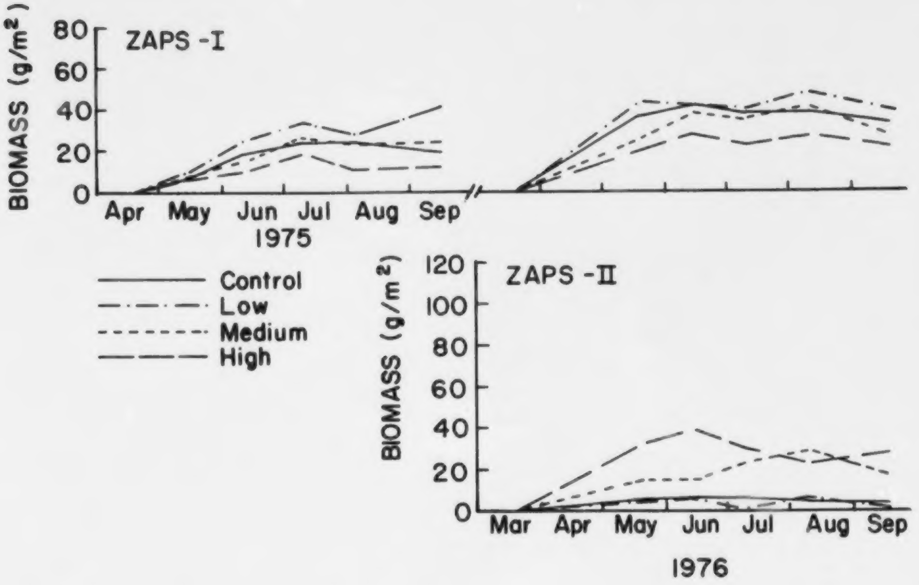

Figure 12.4. Seasonal change in current year's production for prairie Junegrass, ZAPS I and II, 1975 and 1976.

TABLE 12.2. SEASONAL ROOT DYNAMICS* FOR SITES I AND II, 1978.

Date		Site I					Site II			
		Control	Low	Medium	High		Control	Low	Medium	High
20 May 1978	709±27	732±28	759±31	822±38		613±43	766±28	688±34	791±33	
20 July 1978	650±50	614±26	624±24	737±41		643±25	755±38	624±34	608±29	
17 July 1978	549±30	501±27	521±20	491±16		649±27	585±35	546±43	434±22	
16 August 1978	598±35	713±42	741±44	720±32		738±33	765±44	827±37	708±27	
15 September 1978	709±37	771±43	724±31	801±29		646±33	795±46	788±35	665±38	
Average	643	676	674	714		658	733	695	641	

* $\bar{X}$ ± SE, g · m^{-2}, ash-free biomass, 0-10 cm depth.

TABLE 12. 3. SEASONAL AVERAGE OF BELOWGROUND PLANT BIOMASS (TOTAL)* FOR CONTROL AND SO_2 TREATMENTS, SITES I AND II, 1975-1978

Year	Site I				Site II			
	Control	Low	Medium	High	Control	Low	Medium	High
1975	664	601	623	610				
1976	652	619	627	619	616	709	785	717
1978	776	807	797	832	780	873	834	732

* $g \cdot m^{-2}$ ash-free, 0-10 cm depth.

the increases between the first and fourth year were greater with increasing dosages of SO_2, but the reverse was true on Site II by the third year of exposure.

Belowground plant biomass consists of roots, crowns, and rhizomes. The storage organs--crowns and rhizomes--accounted for 6-13% and 3-6% of total belowground plant biomass, respectively, while roots accounted for 83-90% of belowground plant biomass (Table 12.4).

The proportion of total belowground plant biomass in crowns increased during the experiment on both test sites, while that in roots decreased slightly. The increase in crowns appeared to be enhanced by the SO_2 treatments, with the greatest increase taking place on the high treatment of Site I in the fourth year of treatment. A similar trend was exhibited on Site II by the third year of treatment. In addition to the increase of crowns as a proportion of total belowground plant parts, crowns also increased in absolute abundance. By the fourth year on Site I the increases were by factors of 1.5, 1.9, 1.9 and 2.4 on the control, low, medium, and high treatments, respectively. And by the third year on Site II the increases were by factors of 1.4, 1.4, 1.5, and 1.3 for the control, low, medium, and high treatments, respectively.

The proportion of belowground plant biomass existing as rhizomes on Site I increased slightly on the control and decreased on all treated plots (Table 12.4). Site II exhibited no changes in this parameter by the third year of treatment.

In spite of the decrease in rhizomes as a proportion of total belowground organs over the course of the experiment, they did increase in abundance (Table 12.6). By 1978 rhizome biomass had increased by 30%, 20%, 10%, and less than 10% on the control, low, medium, and high treatments, respectively,

TABLE 12.4. MORPHOLOGICAL COMPOSITION* OF BELOWGROUND PLANT BIOMASS (BASED ON SEASONAL AVERAGE ASH-FREE BIOMASS)

Year	Site I				Site II			
	Control	Low	Medium	High	Control	Low	Medium	High
				Rhizomes				
1975	4	4	4	4				
1976	6	5	5	5	4	4	4	4
1978	5	3	3	3	4	4	4	4
				Crowns				
1975	9	9	8	6				
1976	10	11	12	11	11	10	9	11
1978	12	13	12	11	12	12	13	13
				Roots				
1975	86	87	88	90				
1976	83	84	83	84	86	85	87	85
1978	83	84	85	86	84	84	83	83

* %

TABLE 12.5. SEASONAL AVERAGE CROWN BIOMASS* FOR CONTROL AND SO_2 TREATMENTS, SITES I AND II, 1975-1978

Year	Site I				Site II			
	Control	Low	Medium	High	Control	Low	Medium	High
1975	61	56	50	38				
1976	67	68	75	68	65	73	70	76
1978	94	104	95	92	93	103	97	101

* $g \cdot m^{-2}$ ash-free, 0-10 cm depth.

on both study sites. Thus, the reduction in proportional contribution of rhizomes to total belowground plant biomass with increasing SO_2 concentrations resulted largely from a reduction in rates of increase of the rhizome population.

TABLE 12.6. SEASONAL AVERAGE RHIZOME BIOMASS* FOR CONTROL AND SO_2 TREATMENTS, SITES I AND II, 1975-1978

Year	Site I				Site II			
	Control	Low	Medium	High	Control	Low	Medium	High
1975	29	22	25	24				
1976	42	30	29	29	23	32	28	29
1978	39	27	28	26	29	37	32	30

* $g \cdot m^{-2}$ ash-free, 0-10 cm depth.

Aboveground Net Primary Production (ANPP)

ANPP was not estimated for the test sites before exposure to SO_2. Therefore, our assumption that the treatment areas within each of the test sites were equal in production potential to the control areas before fumigation can only be tested with less appropriate information. Harvest estimates of standing crop residual from production occurring in the year prior to fumigation were made in the spring of the first season of exposure and indicated only slight differences among the control and treatment areas within either Site I or Site II (Table 12.7). The low and medium areas on Site I had slightly higher residual material than did the control and high areas. And on Site II the residual material was slightly greater on the control than on the treatment areas. Since slight differences in residuals could also arise from differential weathering during the winter months we conclude that the residual estimates indicate slight, if any differences in production potential within either test site.

TABLE 12.7. RESIDUAL STANDING CROP* OF PREVIOUS YEARS PRODUCTION FOR SITE I AND SITE II IN SPRING BEFORE FIRST SEASON OF SO_2 TREATMENT

	Control	Low	Medium	High
Site I, April 1975	77 ± 7	97 ± 8	93 ± 7	81 ± 10
Site II, March 1976	86 ± 4	75 ± 6	66 ± 6	64 ± 4

* X ± SE.

ANPP ranged from 119 to 268 g · m^{-2} throughout the study period and Site II was more productive than Site I (Table 12.8). The greatest differences in ANPP were between years. While 1977, the driest year of the study (Table 12.1), was clearly the least productive year on both sites, 1978, the wettest year, was the most productive only on Site II. In fact, ANPP in 1978 on Site I was not significantly greater than in 1977 and was lower than ANPP in 1975 and 1976. Since most of the increase in precipitation in 1978 arose from May precipitation being nearly 5 times normal (with 130 mm on May 20), we must conclude that this amount was excessive and reduced productivity via leaching of required nutrients on Site I. Since ANPP was not similarly depressed on Site II we suggest that nutrients were not leached beyond the effective rooting zone because of differences in soil characteristics. Clay contents in the upper 30 cm of the profile range from 33 to 42% on Site II and from 16 to 32% on Site I (Dodd *et al.*, 1979). Fertility factors associated with these textural differences between Site I and Site II are presumed to account for the differential productivity noted between Sites I and II.

TABLE 12.8. ABOVEGROUND NET PRIMARY PRODUCTION* FOR SITES I AND II, 1975-1978†

	ZAPS I				ZAPS II			
Year	Control	Low	Medium	High	Control	Low	Medium	High
	$\overline{X}$ ± SE	$\overline{X}$ ± SE	$\overline{X}$ ± SE	$\overline{X}$ ± SE	$\overline{X}$ ± SE	$\overline{X}$ ± SE	$\overline{X}$ ± SE	$\overline{X}$ ± SE
1975	150±14	149±17	165±13	156±14				
1976	199±13	186± 9	195±11	199± 8	177± 8	218±17	205±14	227±15
1977	126±12	131±13	131± 7	119± 5	137± 7	210±13	169± 9	190±11
1978	136± 8	156±14	123± 6	129± 6	268±18	219±18	214±13	253± 8

* g · m^{-2}.

† ANPP = July current production × 1.18

Treatment differences in ANPP were not detected on Site I. And, although statistically significant treatment differences were noted on Site II, the patterns of the differences are not suggestive of responses to SO_2 exposure.

CONCLUSIONS

Considering the biological and sampling variability associated with our estimates of biomass parameters, we cautiously draw the following conclusions. Aboveground plant biomass dynamics, aboveground net primary productivity, and total belowground biomass dynamics of northern mixed-grass prairie are not immediately sensitive to low level SO_2 exposure regimes. However, short term reductions in rhizome biomass suggest that aboveground biomass dynamics and primary productivity will eventually be altered because of loss of capability of the rhizomes to support aboveground processes in western wheatgrass, the dominant plant species in this grassland type. We assume that similar disruptions of carbon allocation to storage organs of other perennial species also occurs and will ultimately contribute to significant changes in primary productivity, biomass dynamics, and species composition.

REFERENCES

Bartos, D. L., and D. A. Jameson. 1974. A Dynamic Root Model. Am. Midl. Nat., 91:499-504.

Bell, J. N. B., and W. S. Clough. 1973. Depression of Yield in Ryegrass Exposed to Sulfur Dioxide. Nature (London)., 241:47-49.

Bennet, J. H., and A. C. Hill. 1973. Inhibition of Apparent Photosynthesis by Air Pollutants. J. Environ. Qual., 2(4):526-530.

Coupland, R. T. 1973. Producers: I. Dynamics of Aboveground Standing Crop. Canadian IBP Matador Project Tech. Rep. No. 27. Univ. of Saskatchewan, Saskatoon. 159 pp.

Daines, R. H. 1968. Sulfur Dioxide and Plant Responses. J. Occup. Med., 10:216-224.

Davis, C. R., D. R. Howell, and G. W. Morgan. 1966. Sulfur Dioxide Fumigations of Range Grasses Native to Southeastern Arizona. J. Range Manage., 19:60-64.

Detling, J. K., W. P. Parton, and H. W. Hunt. 1979. A Simulation Model of *Bouteloua gracilis* Biomass Dynamics on the North American Shortgrass Prairie. Oecologia, 38:167-191.

Dodd, J. L., W. K. Lauenroth, G. L. Thor, and M. B. Coughenour. 1979. Effects of Chronic Low Level SO_2 Exposures on Producers and Litter Dynamics. In: The Bioenvironmental Impact of a Coal-fired Power Plant, E. M. Preston and T. L. Gullett, eds. 4th Interim Rep., OS/EPA, Corvallis, Oregon. ___ pp.

Dodd, J. L., J. K. Lewis, H. L. Hutcheson, and C. L. Hanson. 1974. Abiotic and Herbage Dynamics Studies on the Cottonwood Site, 1971. US/IBP Grassland Biome Tech. Rep. No. 250. Colorado State Univ., Fort Collins. 195 pp.

Hill, A. C., S. Hill, C. Lamb, and T. W. Barrett. 1974. Sensitivity of Native Desert Vegetation to SO_2 and to SO_2 and NO_2 Combined. J. Air Poll. Cont. Assoc., 24:153-157.

Lauenroth, W. K., K. J. Dodd, R. K. Heitschmidt, and G. R. Woodmansee. 1975. Biomass Dynamics and Primary Production in Mixed Prairie Grasslands in Southeastern Montana: Baseline Data for Air Pollution Studies. Fort Union Coal Field Symposium, Eastern Montana College, Billings. pp. 559-578.

Lauenroth, W. K., and W. C. Whitman. 1971. A Rapid Method for Washing Roots. J. Range Manage., 24:308-309.

Lauenroth, W. K., and W. C. Whitman. 1977. Dynamics of Dry Matter Production in a Mixed-grass Prairie in Western North Dakota. Oecologia, 27:339-351.

Lewis, J. K. 1969. Range Management Viewed in the Ecosystem Framework. In: The Ecosystem Concept in Natural Resource Management, G. M. Van Dyne, ed. Academic Press, New York. pp. 97-187.

Lewis, J. K., J. L. Dodd, H. L. Hutcheson, and C. L. Hanson. 1971. Abiotic and Herbage Dynamics Studies on the Cottonwood Site, 1970. US/IBP Grassland Biome Tech Rep. No. 111. Colorado State Univ., Fort Collins. 147 pp.

Mudd, J. B., and T. T. Kozlowski, eds. 1975. Responses of Plants to Air Pollution. Academic Press, New York. 383 pp.

Northern Great Plains Resource Program. 1975. Effects of Coal Development in the Northern Great Plains. Northern Great Plains Resource Program. (Special report prepared as part of NGPRP in cooperation with federal, state, regional, local and private organizations). 165 pp.

Singh, J. S., W. K. Lauenroth, and R. K. Steinhorst. 1975. Review and Assessment of Various Techniques for Estimating Net Aerial Primary Production in Grasslands from Harvest Data. Bot. Rev., 41:181-230.

Tingey, D. T., R. W. Field, and L. Baird. 1976. Physiological Responses of Vegetation to Coal-fired Power Plant Emissions. In: The Bioenvironmental Impact of a Coal-fired Power Plant: 2nd Interim Rep. Colstrip, Montana, February 1976, R. A. Lewis, N. R. Glass, and A. S. Lefohn, eds. EPA 600/3-76-013, Environmental Protection Agency, Corvallis, Oregon. pp. 14-40.

Treshow, M. 1970. Environment and Plant Response. McGraw-Hill, New York.

SECTION 13

FORAGE QUALITY IN A GRASSLAND EXPOSED TO SULFUR DIOXIDE

D. G. Milchunas, W. K. Lauenroth, and J. L. Dodd

ABSTRACT

Effects of three exposure levels of SO_2 (see section 6) on the nutritive value of western wheatgrass were investigated. Significant increases in plant sulfur content were observed, both with time and level of SO_2 exposure. Plant ash content paralleled the trends observed for sulfur concentrations. Nitrogen levels in western wheatgrass were not affected by SO_2 treatment. The increasing plant sulfur content and decreasing N:S ratios across treatments did not significantly affect forage digestibility as measured by *in vitro* digestible dry matter.

INTRODUCTION

The Northern Great Plains possess a combination of fertile soils and a climate conducive to the production of cereal grains and beef. Gaseous and particulate air pollutants from coal burning pose a threat to traditional resource use because they are phytotoxic and because they disperse over large areas. Data from permit applications (Durran *et al.*, 1979) project an increase in electric generating capacity for the Northern Great Plains from approximately 3000 MW in 1976 to 17000 MW in 1986.

Sulfur dioxide (SO_2) is one product of coal combustion that is of concern with regard to its effects upon plants and animals. Sulfur is an important constituent of many organic molecules, including methionine, cystine, glutathione, thiamine, biotine, and coenzyme-A. At high levels of sulfur exposure, plant yields can be reduced (Bell and Clough, 1973) and increased leaf senescence (Heitschmidt *et al.*, 1977) has been observed. Reports of changes in the amount of plant protein or amino acids in response to SO_2 exposure are controversial (Ziegler, 1975). Microorganisms of the ruminant animal are capable of utilizing N and S in the synthesis of amino acids (Thomas *et al.*, 1951; Starks *et al.*, 1954; Hale and Garrigus, 1953; Abdo *et al.*, 1964). Sulfate supplementation has been shown to increase *in vitro* cellulose digestion (Hunt *et al.*, 1954; Martin *et al.*, 1964; Barton *et al.*, 1971). Sulfur has also been reported to be toxic to rumen microorganisms at a concentration of 100 ppm from sodium sulfate (Hubbert *et al.*, 1958) and at 30 ppm from sodium sulfite (Trinkle *et al.*, 1958). Because SO_2 may be metabolized by the plant and rumen microorganisms and used as a source of sulfur, the balance between positive and negative effects of exposure will depend upon concentration, duration of exposure, and rate of growth and metabolism of SO_2. This study investigates the effects of three exposure levels of SO_2 on the nutritive quality of an important Northern Great Plains forage species, western wheatgrass (*Agropyron smithii* Rydb.).

MATERIALS AND METHODS

Nitrogen and ash were determined by procedures in A.O.A.C. (1965). Total sulfur concentrations were analysed with a Leco Induction Furnace (Laboratory Equipment Corp., St. Joseph, Missouri). *In vitro* digestible dry matter (IVDDM) was determined with techniques described by Tilley and Terry (1964), as modified by Pearson (1970), with buffer solution mixed according to McDougall (1948: Table 11). Inoculum was obtained from a fistulated cow maintained on grass hay for 2 weeks before collection.

Data were analyzed with a repeated measure analysis of variance repeating across time (Winter, 1971). Tukey's Q values were used to identify significant differences between means (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Analysis of sulfur data for western wheatgrass indicated significant across-treatment within-month and across-month within-treatment effects (Table 13.1). No significant differences were observed between the control and low-SO_2 treatments, although a trend of higher sulfur accumulation was evident. The number of significant differences across treatments increased with time of exposure, indicating greater sulfur accumulation across time with higher levels of SO_2 exposure. Significant differences were observed between years on the high treatment, with the order 1975 < 1977 < 1976 (Figure 13.1). We cannot determine, therefore, to what degree sulfur accumulated with each additional year of treatment. Different yearly abiotic factors and plant growth responses appear to outweigh the effect of sulfur accumulation from previous years.

A trend of higher ash concentration with increased level of SO_2 was observed for all year-site combinations (Table 13.2). There also appeared to be a general trend of increasing ash concentrations as the growing season progressed. The nutritional implications of the increased ash content is uncertain. Materna (1961) and Matsushima and Harada (1966) observed increasing potassium content in leaves exposed to SO_2. Incoming SO_2 is rapidly converted to SO_4 by the plant. The formation of sulfate salts for storage of excess sulfur may be increasing the requirement of the plant for the cations used in formation of these salts. At the same time, increased mineral nutrition for consumers may result.

Nitrogen concentrations within-month across-treatments showed no significant differences and no trends were observed (Table 13.3). Values differed significantly on a seasonal basis, with highest levels occurring in May and lowest levels in August or September. Nitrogen levels for site I 1975 and 1977

TABLE 13.1. PERCENT SULFUR CONTENT OF WESTERN WHEATGRASS FOR 5 DATES, 4 TREATMENTS, 2 SITES, AND 3 YEARS OF SO_2 FUMIGATION

Site and treatment	Year	Date					Treatment mean*
		May	June	July	Aug.	Sept.	
Site I	1975						
Control		0.180^{a}	0.098^{a}	0.191^{ab}	0.115^{a}	--	0.141^{a}
Low		0.128^{a}	0.142^{ab}	0.137^{a}	0.134^{a}	--	0.135^{a}
Medium		0.140^{a}	0.207^{bc}	0.241^{bc}	0.262^{b}	--	0.223^{b}
High		0.131^{a}	0.228^{bc}	0.276^{c}	0.331^{b}	--	0.241^{b}
Date mean		0.140	0.169	0.211	0.210	--	0.185
Site I	1976						
Control		0.071^{a}	0.091^{a}	0.094^{a}	0.086^{a}	0.104^{a}	0.089^{a}
Low		0.114^{ab}	0.135^{a}	0.153^{ab}	0.151^{a}	0.174^{a}	0.145^{b}
Medium		0.162^{b}	0.216^{b}	0.218^{b}	0.293^{b}	0.252^{b}	0.288^{c}
High		0.235^{c}	0.242^{b}	0.321^{c}	0.412^{c}	0.364^{c}	0.315^{d}
Date mean		0.146	0.171	0.197	0.235	0.223	0.194
Site I	1977						
Control		0.127^{a}	--	0.113^{a}	0.121^{a}	0.121^{a}	0.146^{a}
Low		0.152^{a}	--	0.133^{a}	0.131^{a}	0.158^{a}	0.152^{a}
Medium		0.163^{a}	--	0.215^{b}	0.213^{b}	0.243^{b}	0.218^{b}
High		0.237^{b}	--	0.270^{b}	0.324^{b}	0.371^{c}	0.290^{c}
Date mean		0.170	--	0.183	0.197	0.223	0.201
Site II	1976						
Control		0.129^{a}	0.107^{a}	0.095^{a}	0.101^{a}	--	0.108^{a}
Low		0.142^{ab}	0.124^{a}	0.167^{a}	0.147^{a}	--	0.145^{a}
Medium		0.213^{b}	0.233^{b}	0.271^{b}	0.277^{b}	--	0.249^{b}
High		0.322^{c}	0.351^{c}	0.433^{c}	0.482^{c}	--	0.397^{c}
Date mean		0.201	0.204	0.241	0.252	--	0.225
Site II	1977						
Control		0.135^{a}	--	0.086^{a}	0.109^{a}	--	0.117^{a}
Low		0.169^{a}	--	0.134^{ab}	0.176^{ab}	--	0.176^{b}
Medium		0.194^{ab}	--	0.177^{b}	0.222^{b}	--	0.189^{b}
High		0.258^{b}	--	0.376^{c}	0.389^{c}	--	0.329^{c}
Date mean		0.189	--	0.195	0.224	--	0.203

* Treatment means for site I 1977 include date for April and an additional August date. Treatment means for site II 1977 include data for April.

abcd Any two means not sharing common superscripts within a column and year-site are significantly different ($P < 0.05$).

were not significantly different, while site I 1976 was significantly lower. Schwartz *et al.* (1978) reported a decrease in crude protein after 2 years of fumigation. Analysis of 3 years of data indicates that the drop in crude protein at site I 1976 was in response to different yearly plant growth patterns as affected by abiotic factors, rather than in response to 2 years of SO_2 exposure. Increases in total nitrogen have been observed when sulfur beyond optimum levels was applied both as fertilizer (Randig, 1956) and SO_2 (Leone and Brennan, 1972). Sulfur accumulation on our high-SO_2 treatment indicates well above optimum levels with no increase in plant total nitrogen.

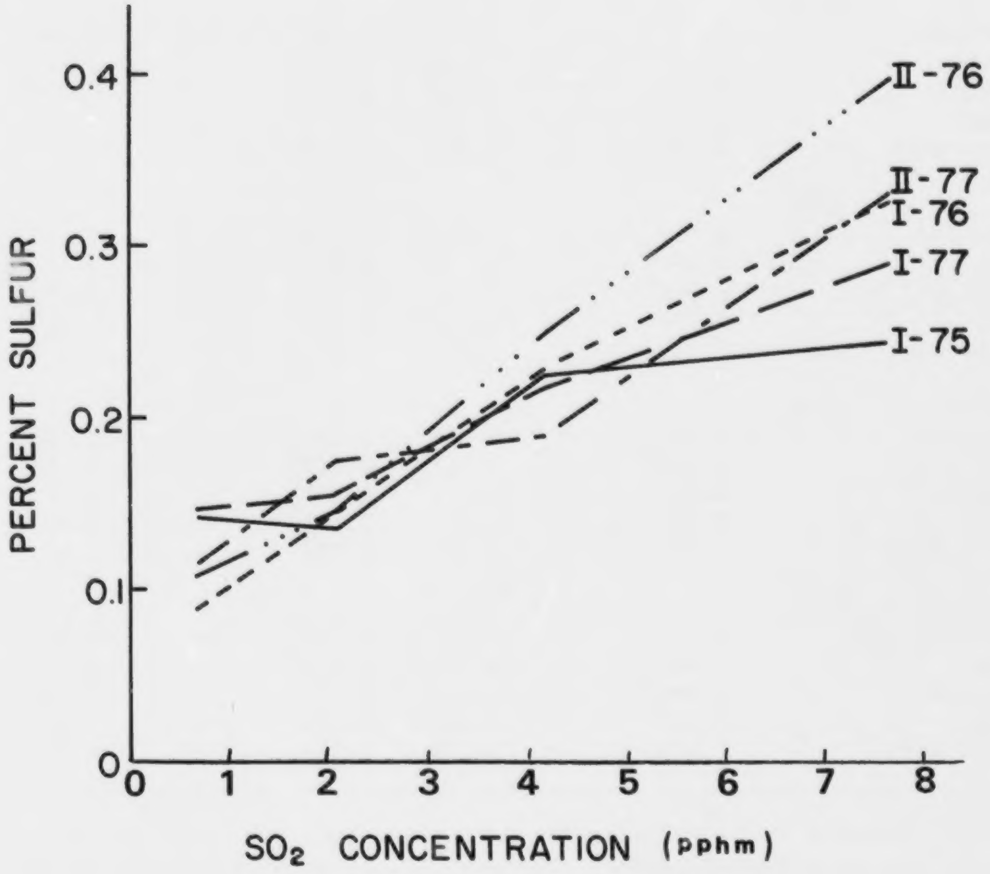

Figure 13.1. Percent sulfur mean across all sampling dates at control, low, medium, and high SO_2 treatments of Site I--1975, 1976, 1977--and Site II--1976, 1977.

TABLE 13.2. PERCENT ASH CONTENT OF WESTERN WHEATGRASS FOR 5 DATES, 4 TREATMENTS, 2 SITES, AND 3 YEARS OF SO_2 FUMIGATION

Site and treatment	Year	Date May	June	July	Aug.	Sept.	Treatment mean*
Site I	1975						
Control		--	7.660	6.782	7.565	--	7.386^{a}
Low		--	7.830	6.380	7.270	--	7.160^{a}
Medium		--	8.070	7.685	7.830	--	7.862^{ab}
High		--	8.210	8.310	8.605	--	8.408^{b}
Date mean		--	7.904	7.289	7.818	--	
Site I	1976						
Control		6.940	6.485	6.570	6.925	7.040	6.972^{a}
Low		7.080	7.385	6.935	7.900	7.795	7.419^{ab}
Medium		7.180	7.130	7.015	8.175	8.295	7.559^{b}
High		8.250	7.890	8.265	9.475	9.280	8.632^{b}
Date Mean		7.363	7.222	7.196	8.119	8.103	
Site I	1977						
Control		6.223	--	7.904	8.461	7.894	7.941^{a}
Low		6.487	--	7.619	8.773	9.474	8.410^{ab}
Medium		6.742	--	7.551	8.350	9.260	8.221^{ab}
High		7.832	--	8.137	9.209	9.615	8.828^{b}
Date mean		6.822	--	7.803	8.698	9.061	
Site II	1976						
Control		9.020	7.620	8.605	8.265	7.780	8.258^{ab}
Low		8.265	6.420	8.400	7.440	8.675	7.840^{a}
Medium		9.575	8.035	9.020	9.045	8.610	8.884^{bc}
High		9.425	8.205	9.160	9.325	10.480	9.190^{c}
Date mean		9.071	7.570	8.796	8.519	8.667	
Site II	1977						
Control		7.532	--	8.298	8.632	--	8.088^{ab}
Low		6.466	--	6.944	7.651	--	7.258^{a}
Medium		7.147	--	8.307	8.533	--	7.787^{ab}
High		7.250	--	8.256	8.843	--	8.374^{b}
Date mean		7.099	--	7.977	8.415	--	

* Treatment means for site I 1977 include April and an additional August sampling date.

abc Any two means not sharing common superscripts within a column and year-site are significantly different ($P < 0.05$).

TABLE 13.3. PERCENT NITROGEN CONTENT OF WESTERN WHEATGRASS FOR 5 DATES, 4 TREATMENTS, 2 SITES, AND 3 YEARS OF SO_2 FUMIGATION

Site and treatment	Year	Date					Treatment mean*
		May	June	July	Aug.	Sept.	
Site I	1975						
Control		2.545	1.821	1.333	1.107	--	1.701
Low		2.513	1.721	1.269	1.082	--	1.646
Medium		2.694	1.677	1.361	1.124	--	1.574
High		2.662	1.944	1.183	1.000	--	1.697
Date mean		2.590^{a}	1.791^{b}	1.286^{c}	1.078^{d}	--	1.657
Site I	1976						
Control		2.230	1.544	1.237	0.974	0.760	1.497
Low		2.177	1.378	1.059	0.799	0.640	1.353
Medium		2.047	1.461	1.094	0.936	0.646	1.385
High		1.891	1.351	1.092	0.890	0.685	1.306
Date mean		2.086^{a}	1.434^{b}	1.120^{c}	0.900^{d}	0.683^{d}	1.385
Site I	1977						
Control		2.208	1.979	1.384	1.270	0.944	1.710
Low		2.313	1.837	1.087	1.096	0.700	1.583
Medium		2.094	1.897	1.186	1.092	0.862	1.567
High		2.527	1.960	1.218	1.237	0.862	1.736
Date mean		2.285^{a}	1.918^{b}	1.219^{c}	1.174^{c}	0.842^{d}	1.649
Site II	1976						
Control		2.285	1.099	1.377	1.035	0.984	1.449
Low		2.446	1.908	1.599	1.153	0.949	1.776
Medium		2.303	1.644	1.322	1.012	0.811	1.570
High		2.498	1.915	1.476	1.175	1.298	1.766
Date mean		2.383^{a}	1.641^{b}	1.443^{b}	1.094^{c}	0.996^{c}	1.640
Site II	1977						
Control		2.587	2.212	1.580	1.729	--	2.027
Low		2.950	2.406	1.590	1.699	--	2.161
Medium		2.770	2.279	1.570	1.498	--	2.095
High		2.815	2.459	1.603	1.907	--	2.196
Date mean		2.781^{a}	2.339^{b}	1.588^{c}	1.708^{c}	--	2.121
All sites	All years						
Control		2.371	1.731	1.382	1.223	--	1.677
Low		2.480	1.850	1.321	1.165	--	1.704
Medium		2.347	1.791	1.277	1.132	--	1.628
High		2.478	1.925	1.314	1.242	--	1.740
Date mean		2.421	1.824	1.325	1.191	--	1.688

* September data are not included in analysis of variance or treatment mean.

abcd Any two means not sharing a common superscript within a row are significantly different ($P < 0.05$).

Cattle and sheep require a N:S ratio of 11-13:1 and rumen microorganisms of 12-14:1 (Leibholz and Naylor, 1971; Moir *et al.*, 1967; Whanger *et al.*, 1978). This is similar to the ratio found in many proteins (Block and Weiss, 1956). N:S ratios (Table 13.4) suggest that sulfur may be limiting protein production on control and low-SO_2 fumigation plots in early spring. Whether this occurs cannot be ascertained from total nitrogen (crude protein) analysis. Plants contain nitrogen in various forms; therefore, true protein nitrogen to non-protein nitrogen ratios would be necessary before conclusions could be drawn about a possible fertilizing effect of SO_2. Plant senescence observed from SO_2 exposure (Heitschmidt, 1977) may in part be earlier maturation in response to increased spring protein production made possible by the increase in available sulfur, and this may occur irrespective of soil sulfur levels. Although roots apparently have the ability to reduce sufficient sulfate to provide the sulfide for their own needs, they do not translocate appreciable amounts of reduced sulfur forms to the shoots (Salesbury and Ross, 1969). Therefore, the differential requirements for reduced sulfur forms and thereafter ability of leaves to reduce incoming sulfur could be compensated for by stomatal entry of SO_2.

Although SO_2 exposure in early spring may have a fertilizing effect, there are data indicating a reduction in soluble protein (Rabe and Kreeb, 1979) and changes of free amino acid concentrations (Godzik and Linskens, 1974) with SO_2 fumigation when soil sulfur levels are adequate. Longer exposure periods always resulted in a decrease in true protein concentration. These studies were conducted with crop species and the length of SO_2 treatment was relatively short compared with our May through October exposure schedule. Our data indicated that the effect of SO_2 exposure on protein production in native range plants cannot be determined from total nitrogen analysis. True protein and non-protein forms of nitrogen need to be distinguished.

The high N:S ratios in May and the high sulfur concentrations in August and September did not significantly affect digestibility of western wheatgrass when measured by the *in vitro* digestible-dry-matter technique (Table 13.5). However, one cannot conclude that the SO_2 treatments do not affect ruminant metabolism of protein or energy. The disappearance of dry matter or cellulose is only of indirect importance to the energy metabolism of ruminants. The products produced from this disappearance supply the ruminant's energy requirements and are of more practical importance. The proportion and quantity of these products vary with the array of substrate components fermented. Huisingh *et al.* (1974) described high concentrations of sulfate-reducing bacteria in the rumen of sheep given sulfate as the only S source. Sulfur in the ruminant diet can alter microbial population dynamics and, because of the different end products supplied to the host by various species of microorganisms, may thereby affect energy as well as nitrogen metabolism.

SO_2 effects on ruminant nutrition may be manifested in structural fiber, carbohydrate, protein-nitrogen, and sulfur status of the plant and thus influence the rumen microbial ecosystem and the supply of energy and protein to the host. The content of a particular element or dietary constituent in a forage has little significance unless it is qualified by a factor indicating the biological utilization of that component. Because of the complexity of

TABLE 13.4. NITROGEN TO SULFUR RATIOS OF WESTERN WHEATGRASS FOR 5 DATES, 4 TREATMENTS, 2 SITES, AND 3 YEARS OF SO_2 FUMIGATION

Site and treatment	Year	Date					Treatment mean*
		May	June	July	Aug.	Sept.	
Site I	1975						
Control		14.14	18.58	6.98	9.63	--	12.06
Low		19.63	12.12	9.26	8.07	--	12.19
Medium		19.24	8.10	5.65	4.29	--	7.06
High		20.32	8.53	4.29	3.02	--	7.04
Date mean		18.50	10.60	6.09	5.13	--	8.96
Site I	1976						
Control		31.41	16.97	13.16	11.33	7.31	16.82
Low		19.10	10.21	6.92	5.29	3.68	9.33
Medium		12.64	6.76	5.02	3.19	2.56	6.07
High		8.05	5.58	3.40	2.16	1.88	4.15
Date		14.29	8.39	5.69	3.83	3.06	4.14
Site I	1977						
Control		17.39	--	12.25	10.50	7.80	11.71
Low		15.22	--	8.17	8.37	4.43	10.41
Medium		12.85	--	5.52	5.13	3.55	7.19
High		10.66	--	4.51	3.82	2.32	5.99
Date mean		13.44	--	6.66	5.96	3.78	8.20
Site II	1976						
Control		17.71	10.27	14.49	10.25	--	13.42
Low		17.23	15.39	9.57	7.84	--	12.25
Medium		10.81	7.06	4.88	3.65	--	6.31
High		7.76	5.46	3.41	2.44	--	4.45
Date mean		11.36	8.04	5.99	4.34	--	7.29
Site II	1977						
Control		19.16	--	18.37	15.86	--	17.32
Low		10.77	--	11.87	9.65	--	12.28
Medium		14.28	--	8.87	6.75	--	11.08
High		10.91	--	4.26	4.90	--	6.67
Date mean		14.71	--	8.14	7.63	--	10.45

* For May, June, July, and August data.

TABLE 13.5. PERCENT *IN VITRO* DIGESTIBLE DRY MATTER OF WESTERN WHEATGRASS FOR 7 DATES, 4 TREATMENTS, 2 SITES, AND 3 YEARS OF SO_2 FUMIGATION

Site and treatment	Year	Date April	 May	 June	 July	 Aug. 6	 Aug. 30	 Sept.	Treatment mean*
Site I	1975								
Control		--	71.84	54.18	50.57	45.73	--	40.67	50.46^{a}
Low		--	64.40	53.92	47.05	44.41	--	34.10	49.46^{a}
Medium		--	69.20	55.53	50.72	44.24	--	39.43	49.90^{a}
High		--	69.04	60.04	51.92	45.91	--	37.32	51.91^{a}
Date mean		--	67.86^{a}	55.92^{b}	50.07^{c}	45.07^{d}	--	38.05^{e}	50.42
Site I	1976								
Control		--	62.58	49.40	47.81	41.75	--	36.97	47.70^{a}
Low		--	62.08	48.50	44.05	41.75	--	36.23	46.52^{a}
Medium		--	59.97	50.46	44.44	40.83	--	37.30	46.60^{a}
High		--	58.20	50.63	44.96	38.68	--	35.54	45.60^{a}
Date mean		--	60.71^{a}	49.75^{b}	45.32^{c}	40.75^{b}	--	36.51^{e}	46.61
Site I	1977								
Control		83.17	72.37	71.20	60.95	55.70	51.41	54.10	64.73^{a}
Low		83.70	70.89	--	58.31	55.51	52.03	47.76	59.00^{a}
Medium		83.60	69.00	--	57.98	53.15	53.65	53.12	61.13^{a}
High		83.08	74.08	67.09	58.12	56.92	50.98	52.82	61.57^{a}
Date mean		83.38^{a}	71.46^{b}	69.15^{b}	58.86^{c}	55.23^{d}	52.05^{d}	51.86^{d}	61.75
Site II	1976								
Control		--	60.52	56.06	48.53	42.84	--	38.99	49.39^{a}
Low		--	66.89	54.34	50.74	45.05	--	40.02	51.41^{a}
Medium		--	66.70	52.72	50.15	43.32	--	34.98	49.57^{a}
High		--	69.18	54.61	47.90	46.49	--	41.12	52.06^{a}
Date mean		--	65.82^{a}	54.43^{b}	49.33^{c}	44.43^{d}	--	39.03^{e}	50.61
Site II	1977								
Control		84.75	73.46	--	65.93	64.36	--	--	70.32^{a}
Low		84.20	74.88	--	63.41	61.23	--	--	70.93^{a}
Medium		82.30	71.43	--	64.50	62.03	--	--	69.22^{a}
High		82.14	72.63	--	63.82	61.65	--	--	70.06^{a}
Date mean		83.29^{a}	73.10^{b}	--	64.40^{c}	62.32^{c}	--	--	70.20

* For May, June, July, and August data.

abcde Any two means not sharing a common superscript within a row are significantly different ($P < 0.05$).

interactions and possible masking effects, the study of the effect of SO_2 on forage nutritive value simply by qualifying plant components and digestible dry matter or cellulose disappearance is inadequate.

CONCLUSIONS

Increased sulfur accumulation was observed in western wheatgrass both across time and level of SO_2 exposure. Trends in ash concentration paralleled those of sulfur. Nitrogen concentrations decreased during the growing season but were not affected by SO_2 treatments. Nitrogen to sulfur ratios were higher than optimum in May on the control and low SO_2 treatments and very low on the high SO_2 treatments in August and September. These ratios suggest a possible fertilizing effect of SO_2 in early spring, with toxic levels being reached in fall. SO_2 fumigation, and the high forage sulfur concentration, did not offset digestibility for ruminants as measured *in vitro*.

REFERENCES

Abdo, K. M., K. W. King, and R. W. Engel. 1964. Protein Quality of Rumen Microorganisms. J. Anim. Sci., 23:734.

A.O.A.C. 1965. Official Methods of Analysis, 10th ed. Ass. Offic. Agr. Chem., Washington, D. C.

Barton, J. S., L. W. Bull, and R. W. Henten. 1971. Effects of Various Levels of Sulfur upon Cellulose Digestion in Purified Diets and Lignocellulose Digestion in Corn Fodder Pellets *in vitro*. J. Anim. Sci., 33:682.

Bell, J. N. B., and W. S. Clough. 1973. Depression of Yield in Ryegrass Exposed to Sulfur Dioxide. Nature, 241:47.

Block, R. J., and K. W. Weiss. 1956. Amino Acid Handbook. Thomas, Springfield, Illinois.

Durran, D. R., M. J. Meldgin, M. K. Liu, T. Thoem, and D. Henderson. 1979. A Study of Long Range Air Pollution Problems Related to Coal Development in the Northern Great Plains. Atmos. Environ., 13:1021-1037.

Godzik, S., and H. F. Linskens. 1974. Concentration Changes of Free Amino Acids in Primary Bean Leaves After Continuous and Interrupted SO_2 Fumigation and Recovery. Environ. Pollut., 7:25.

Hale, W. H., and U. S. Garrigus. 1953. Synthesis of Cystine in Wool from Elemental Sulfur and Sulfate Sulfur. J. Anim. Sci., 12:492.

Heitschmidt, R. K. 1977. Chronic Effects of SO_2 on Western Wheatgrass in a Montana Grassland. Ph.D. Thesis. Colorado State Univ., Fort Collins. 100 pp.

Hubbert, F., Jr., E. Cheng, and W. Burroughs. 1958. Mineral Requirements of Rumen Microorganisms for Cellulose Digestion *in vitro*. J. Anim. Sci., 17:559.

Huisingh, J., J. J. McNeill, and G. Matrone. 1974. Sulfate Reduction by a *Desulforibrio* Species Isolated from Sheep Rumen. Appl. Microbiol., 28:489.

Hunt, C. H., O. G. Bentley, T. V. Hershberger, and J. H. Cline. 1954. The Effect of Carbohydrates and Sulfur on B-vitamin Synthesis, Cellulose Digestion, and Urea Utilization by Rumen Microorganism *in vitro*. J. Anim. Sci., 13:570.

Leibholz, J., and R. W. Naylor. 1971. The Effect of Urea in the Diet of the Early-weaned Calf on Weight Gain, Nitrogen and Sulfur Balance, and Plasma Urea and Free Amino Acid Concentrations. Aust. J. Agric. Res., 22:655.

Martin, J. E., L. R. Arrington, J. E. Moore, C. B. Ammerman, G. K. Davis, and R. L. Shirley. 1964. Effect of Magnesium and Sulfur upon Cellulose Digestion of Purified Rations by Cattle and Sheep. J. Nutr., 83:60.

Materna, J. 1961. Einfluss des schwefeldioxyds auf die mineralische zusammensetzung der fichtennadeln. Naturwissenschaften, 48:723-724.

Matsushima, J., and M. Harada. 1966. Sulfur Dioxide Gas Injury to Fruit Trees. V. Absorption of Sulfur Dioxide by Citrus Trees and Its Relation to Leaf Fall and Mineral Contents of Leaves. J. Jap. Soc. Hort. Sci., 35:242-246.

McDougall, E. I. 1948. Studies on Ruminant Saliva. I. The Composition and Output of Sheep's Saliva. Biochem. J., 43:99.

Moir, R. J., M. Somers, and A. C. Bray. 1967. Utilization of Dietary Sulfur and Nitrogen. Sulfur Institute J., 3:15.

Pearson, H. A. 1970. Digestibility Trials: *In vitro* Techniques. In: Range and Wildlife Habitat Evaulation--A Research Symposium, USDA Misc. Pub. No. 1147. pp. 85-92.

Rabe, R., and K. H. Kreeb. 1979. Enzyme Activities and Chlorophyll and Protein Content in Plants as Indicators of Air Pollution. Environ. Pollut., 19:119.

Salesbury, F. B., and C. Ross. 1969. Metabolism and Functions of Nitrogen and Sulfur. In: Plant Physiology. Wadsworth Publ. Co., Calif. 747 pp.

Schwartz, C. C., W. K. Lauenroth, R. K. Heitschmidt, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulphur Dioxide on the Nutrient Quality of Western Wheatgrass. J. Appl. Ecol., 15:869.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State Univ. Press, Ames. 593 pp.

Starks, P. B., W. H. Hale, U. S. Garrigus, R. B. Forbes, and M. F. James. 1954. Response of Lambs Fed Various Levels of Elemental Sulfur, Sulfate Sulfur and Methionine. J. Anim. Sci., 13:249.

Thomas, W. E., J. K. Loosli, H. H. Williams, and L. A. Maynard. 1951. The Utilization of Inorganic Sulfate and Urea Nitrogen by Lambs. J. Nitr., 43:515.

Tilley, J. M. A., and A. Terry. 1963. A Two-stage Technique for the *in vitro* Digestion of Forage Crops. J. Br. Grassland Soc., 18:104.

Trinkle, A., E. Cheng, and W. Burroughs. 1958. Availability of Different Sulfur Sources for Rumen Micro-organisms in *in vitro* Cellulose Digestion. J. Anim. Sci., 17:1191 (abstr.).

Whanger, P. D., P. H. Weswig, and J. E. Oldfield. 1978. Selenium, Sulfur and Nitrogen Levels in Rumen Microorganisms. J. Anim. Sci., 46:515.

Winter, B. J. 1971. Statistical Principles in Experimental Design, 2nd ed. McGraw-Hill, New York.

Ziegler, I. 1975. The Effect of SO_2 Pollution on Plant Metabolism. Residue Rev., 56:79.

SECTION 14

CHLOROPHYLL REDUCTION IN WESTERN WHEATGRASS EXPOSED TO SULFUR DIOXIDE

W. K. Lauenroth and J. L. Dodd

ABSTRACT

Chlorophyll concentrations in the dominant species in the grassland, western wheatgrass, were examined under exposure to controlled SO_2 concentrations. Significant decreases in chlorophylls *a* and *b* were observed in the absence of visible necrosis. Chlorophyll *a* was more sensitive than chlorophyll *b*. Sensitivity of chlorophylls to SO_2 changed as the growing season progressed indicating cumulative effects and interactions with normal senescence.

INTRODUCTION

Early recognition of chlorosis as a symptom of sulfur dioxide exposure led investigators to suspect interactions of the gas with chlorophyll metabolism. Experimental work with lichens (Rao and LeBlanc, 1965) and bryophytes (Coker, 1967) confirmed that exposure to high concentrations of SO_2 results in the breakdown of chlorophylls to phaeophytin. Gilbert (1968), using a concentration which one may expect in polluted areas (1.0 pphm SO_2), found that sensitive lichen species underwent a continual decline in chlorophyll content over 40 days, while resistant species were unaffected. Malhotra (1977) exposed pine needles to aqueous SO_2 and found significant decreases in chlorophyll content with SO_2 concentrations between 10,000 and 50,000 pphm. Chlorophyll *a* was more sensitive than chlorophyll *b*.

SO_2 exposure can cause quantitative changes in chlorophyll content. Most studies rely upon leaf injury to express the amount of chlorosis or necrosis caused by SO_2 as well as other phytotoxic pollutants. Knudson *et al.* (1977) found a good relationship between chlorophyll reduction and necrosis and chlorosis for pinto beans exposed to ozone. They concluded that chlorophyll reduction measured the same aspect of leaf injury as visual estimation but with more precision and without observer bias. Using chlorophyll content provides the potential for developing relationships with photosynthesis, photosynthetic efficiency, and production.

We investigated the impact of three controlled SO_2 treatments, under field conditions, on the chlorophyll content of western wheatgrass (*Agropyron smithii* Rydb.). Our objective was to determine if chlorophyll destruction was occurring in the absence of visible necrosis.

MATERIALS AND METHODS

Plant material was collected to allow us to measure chlorophyll contents of individual leaves as well as entire tillers (live plus dead leaves). Leaf chlorophyll content was measured for leaves of three different developmental ages on each date. A sample for leaf chlorophyll measurement consisted of two leaves representing the same developmental age that had been cut, at the ligule, from two different tillers. On each measurement date, 10 such samples were collected for each of the three leaf ages from each SO_2 treatment. Tiller chlorophyll content was estimated by collecting and analyzing 10 tillers from each treatment on 9 July and 20 tillers per treatment on 8 and 18 August.

Chlorophyll analysis procedures followed Knudson, *et al.* (1977), with the following modifications. Immediately after collection, leaves were cut in approximately 2-mm segments and placed in a scintillation vial (approximately 30 ml) containing 100% ethanol. The vials were then kept in the dark for 48 hrs. The contents of each vial were then placed in a blender with an additional 30 ml of ethanol (one rinse) and blended for 1 min. The contents of the blender were poured into a 250-ml flask, capped, and stored in the dark for an additional 24 hr. to complete the extraction. The ethanol-chlorophyll solution was filtered through Whatman No. 540 filter paper, which had been oven-dried at 60°C for 12 hr. and weighed. Each filter paper was then placed back in the oven for 12 hr. and the volume of the ethanol-chlorophyll solution standardized and absorbances were measured at 665 and 649 nm with a spectrophotometer. Chlorophyll *a* and *b* contents were calculated using the equations presented in Knudson, *et al.* (1977). Residual dry weights were determined by reweighing the filter papers, and chlorophyll content is expressed as μg chlorophyll $\cdot$ mg dry weight^{-1}. Analysis of the change in dry weight of western wheatgrass leaves after the ethanol extraction indicated losses of 16 to 19 percent with no differential influence of SO_2 exposure. Chlorophyll accounted for less than 1 percent of this loss.

Statistical analysis of the results was by analysis of variance with Tukey's Q values used to compare means (Snedecor and Cochran, 1967). All references to differences in chlorophyll concentrations in the results section are statistically significant at the 5 percent probability level.

RESULTS AND DISCUSSION

Analysis of chlorophyll *a* and *b* concentrations in leaves of western wheatgrass exposed to three controlled SO_2 concentrations resulted in significant differences due to date, treatment, and leaf age, as well as the two- and three-way interactions of these factors.

Maximum chlorophyll concentrations ranging from 4 to 9 $\mu g \cdot mg^{-1}$ were measured on the May collection date (Figures 14.1 and 14.2). June through August chlorophyll concentrations were in the 1 to 3 $\mu g \cdot mg^{-1}$ range. Rauzi

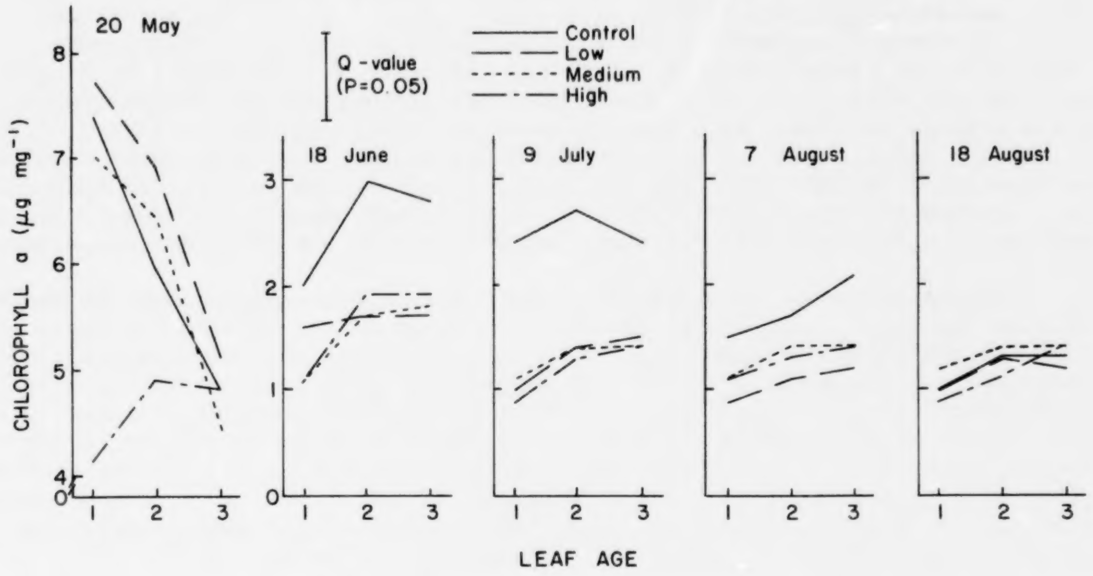

Figure 14.1. Chlorophyll *a* concentration of western wheatgrass leaves exposed to three controlled SO_2 treatments.

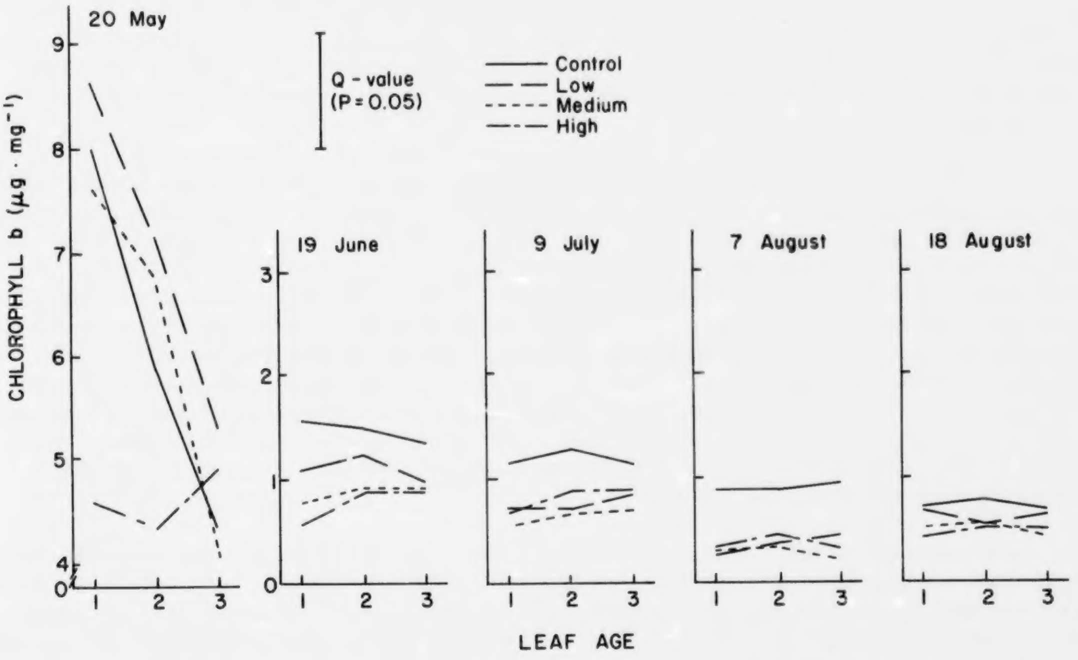

Figure 14.2. Chlorophyll *b* concentration of western wheatgrass leaves exposed to three controlled SO_2 treatments.

and Dobrenz (1970) reported peak chlorophyll *a* and *b* concentrations of oven-dried western wheatgrass tillers of 1.5 and 1.1 $\mu g \cdot mg^{-1}$. Bokhari (1976) found chlorophyll contents of western wheatgrass plants grown in a growth chamber to vary from 4 to 6 $\mu g \cdot mg^{-1}$ for chlorophyll *a* and from 1 to 2 $\mu g \cdot mg^{-1}$ for chlorophyll *b*. We did not encounter differences between chlorophyll *a* and *b* until mid-June, when they amounted to only 1 $\mu g \cdot mg^{-1}$. Bokhari (1976) reported a chlorophyll *a*:*b* ratio of three for western wheatgrass over a 100-day developmental period. We found the ratio to be near one in May. It increased to a maximum of approximately three in early August and dropped back to two in mid-August. Sanger (1971) reported similar dynamics for three deciduous trees.

Concentrations of chlorophylls *a* and *b* were significantly lower on the May date in the high SO_2 treatment for the two oldest leaves than in the control or other treatments (Figures 14.1 and 14.2). In June, the chlorophylls had decreased to approximately 20% of the May values. While chlorophyll *b* concentrations were not significantly reduced by SO_2 after May, significant differences in chlorophyll *a* concentrations were found on all except the 18 August sample date. That chlorophyll *a* was more sensitive to SO_2 than chlorophyll *b* is in agreement with the findings of Malhotra (1977). He demonstrated that the decrease in chlorophyll *a* in pine needles treated with aqueous SO_2 was the result of the conversion of chlorophyll to phaeophytin.

On 18 June, chlorophyll *a* concentration in leaves from control plants was greater than that measured for leaves from either the medium or high treatment, regardless of leaf age (Figure 14.1). Chlorophyll *a* concentration in leaves from the control was also greater than that for leaves from the low treatment except for the oldest leaf. In July, leaves from the control treatment have significantly greater chlorophyll *a* concentration than leaves from the other treatments, regardless of age. In early August the only significant difference that remained was between the youngest leaves from the control and low treatment. By 18 August chlorophyll *a* concentrations were similar for all treatments.

The data for both chlorophylls indicated a pattern that began in May with similarity among the control and low and medium treatments. By June the medium treatment was much more similar to the high treatment. In July all three SO_2 treatments were similar and, in the case of chlorophyll *a*, much lower in concentration than the control. By early August chlorophyll *a* concentrations had reached a minimum. In addition, there was a pattern of maximum differences in chlorophyll concentrations among the oldest leaves at the beginning of the growing season, indicating a cumulative effect of SO_2. By the middle of the growing season the differences were found among all leaves with similar magnitudes, indicating no cumulative effect. By late in the growing season the final significant difference is between youngest leaves, suggesting perhaps inhibition of chlorophyll synthesis.

Analysis of total tiller chlorophyll content (live plus dead leaves) resulted in no significant effects due to treatments. Combination of these data with estimates of western wheatgrass tiller density and biomass for the July sample date after correction for dry matter losses in the extraction procedure made it possible for us to calculate chlorophyll standing crop (Table 14.1). Western wheatgrass tiller density (tillers per m^2) and biomass ($g \cdot m^{-2}$) were available from a concurrent investigation of the impact of SO_2 on grassland primary production (Dodd *et al.*, 1979). Chlorophyll content on an individual tiller basis was not very different among treatments, although plants from the high treatment had the lowest amount of both chlorophylls. Estimates of chlorophyll standing crop indicated 10 to 20 $mg \cdot m^{-2}$ differences in chlorophyll *a* between the control and treatment plots and 7 to 10 $mg \cdot m^{-2}$ differences in chlorophyll *b* (Table 14.1). Taylor and Leininger (1978) reported a gradient in infra-red reflectance of the SO_2 treated plots that corresponded to increasing SO_2 concentrations. The observed gradients in chlorophyll concentrations per tiller and chlorophyll standing crop were in agreement with the photographic data.

Heitschmidt *et al.* (1978) found no visible injury to western wheatgrass exposed to SO_2. The only detectable responses of western wheatgrass to SO_2 were increased sulfur (Lauenroth *et al.*, in press) and decreased functional leaf life (Heitschmidt *et al.*, 1978). Chlorophyll analyses from the same experimental area support those results and demonstrate that biochemical interactions involving SO_2 are occurring within the leaf before their expression as tissue death (Ziegler,1975). These results complicate the widely accepted relationship between classification of a plant sensitive to SO_2 and visible leaf injury (Thomas *et al.*, 1950; Kondo and Sugahara, 1978).

TABLE 14.1. STANDING CROP OF CHLOROPHYLLS *A* AND *B* IN WESTERN WHEATGRASS

	Chlorophyll			
	Concentration *a*		Concentration *b*	
Treatment	($mg \cdot plant^{-1}$)	($mg \cdot m^{-2}$)	($mg \cdot plant^{-1}$)	($mg \cdot m^{-2}$)
Control	0.10	48	0.06	28
Low	0.11	43	0.06	22
Medium	0.11	31	0.05	15
High	0.09	41	0.05	21

Does a reduction in chlorophyll concentration necessarily result in a reduction in photosynthesis? Malhotra (1977) found that the breakdown in chlorophyll molecules by SO_2 was reflected in a decreased ability of pine needles to photosynthesize, as measured by $H^{14}CO_3$ fixation. This decline in photosynthesis may be related to an inactivation of the functional sulf-hydrol groups of enzymes required for fatty acid biosynthesis. Glycero-lipids have been reported as important constituents of chloroplasts, mitochondria, and other organelles (Macher *et al.*, 1975).

Brougham (1960) found a significant relationship between maximum rates of dry-matter accumulation of several species and chlorophyll content of the portion of the stand canopy exposed to light intensities greater than 500 f.c. at noon. Hesketh (1963) reported that chlorophyll content was not an important explanatory variable for differences in photosynthetic rates among species. Ruetz (1973) and Buttery and Buzzel (1977) both found significant positive relationships between leaf chlorophyll content and photosynthesis for a single plant species. Although one would expect many environmental controls operating to modify photosynthetic rates of western wheatgrass, it is possible that a reduction in chlorophyll concentration as a result of SO_2 exposure could reduce their photosynthetic capacity.

CONCLUSIONS

Chlorophyll concentrations in western wheatgrass leaves is a sensitive indicator of exposure to SO_2. Reductions in chlorophyll concentrations may result in undetectable decreases in net primary production.

REFERENCES

Bokhari, U. G. 1976. Influence of Temperatures, Water Stress, and Nitrogen Treatments on Chlorophyll and Dry Matter of Western Wheatgrass. J. Range Manage., 29:127-131.

Brougham, R. W. 1960. The Relationship Between the Critical Leaf Area, Total Chlorophyll Content, and Maximum Growth-rate of Some Pasture and Crop Plants. Ann. Bot. N.S., 24:463-474.

Buttery, B. R., and R. I. Buzzel. 1977. The Relationship Between Chlorophyll Content and Rate of Photosynthesis in Soybeans. Can. J. Plant Sci., 57:1-5.

Coker, P. D. 1967. The Effects of SO_2 Pollution on Bark Epiphytes. Trans. Br. Bryo. Soc., 5:341.

Dodd, J. L., W. K. Lauenroth, G. L. Thor, and M. B. Coughenour. 1979. Effects of Chronic Low Level SO_2 Exposure on Producers and Litter Dynamics. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-049. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 384-493.

Gilbert, O. L. 1968. Biological Indications of Air Pollution. Ph. D. Thesis, University of Newcastle-on-Tyne.

Heitschmidt, R. K., W. K. Laurenroth, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulphur Dioxide on Western Wheatgrass in a Southeastern Montana Grassland. J. Appl. Ecol., 14:693-702.

Hesketh, J. D. 1963. Limitations to Photosynthesis Responsible for Differences Among Species. Crop. Sci., 3:493-496.

Knudson, L. L., T. W. Tibbits, and G. E. Edwards. 1977. Measurement of Ozone Injury by Determination of Leaf Chlorophyll Concentration. Plant Physiol., 60:606-608.

Kondo, N., and K. Sugahara. 1978. Changes in Transpiration Rate of SO_2-Resistant and -Sensitive Plants with SO_2 Fumigation and the Participation of Abscisic Acid. Plant Cell Physiol., 19:365-373.

Lauenroth, W. K., D. Bicak, and J. L. Dodd. In press. Sulfur Accumulation in Western Wheatgrass Exposed to Three Sulfur Concentrations. Plant and Soil.

Macher, B. A., C. P. Brown, T. T. McManus, and J. B. Mudd. 1975. Studies on Phospholipid-synthesizing Enzyme Activities During the Growth of Etiolated Cucumber Cotyledons. Plant Physiol., 55:130-136.

Malhotra, S. S. 1977. Effects of Aqueous Sulphur Dioxide on Chlorophyll Destruction in *Pinus contorta*. New Phytol., 78:101-109.

Rao, D. N., and F. LeBlanc. 1965. Effects of SO_2 on the Lichen Algae with Special Reference to Chlorophyll. Bryologist, 69:69.

Rauzi, F., and A. K. Dobrenz. 1970. Seasonal Variation of Chlorophyll in Western Wheatgrass and Blue Grama. J. Range Manage., 23:372-373.

Ruetz, W. F. 1973. The Seasonal Pattern of CO_2 Exchange of *Festuca rubra* L. in a Montane Meadow Community in Northern Germany. Oecologia (Berl.), 13:247-269.

Sanger, J. E. 1971. Quantitative Investigations of Leaf Pigments from their Inception in Buds Through Autumn Coloration to Decomposition in Falling Leaves. Ecology, 52:1075-1089.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State Univ. Press. Ames.

Taylor, J. E., and W. C. Leininger. 1978. Remote Sensing of the Bioenvironmental Effects of Stack Emissions in the Colstrip Vicinity. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-201. U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 280-290.

Thomas, M. D., R. H. Hendricks, and G. R. Hill. 1950. Sulfur Metabolism in Plants: Effects of SO_2 on Vegetation. Ind. Eng. Chem., 42:2231-2235.

Ziegler, I. 1975. The Effect of SO_2 Pollution on Plant Metabolism. Residue Rev., 56:79-105.

SECTION 15

RESPONSE OF A GRASSLAND TO SULFUR AND NITROGEN TREATMENTS UNDER CONTROLLED SO_2 EXPOSURE

W. K. Lauenroth and J. L. Dodd

ABSTRACT

Interactions of nitrogen and sulfur fertilization and SO_2 exposure were examined in a native grassland. Nitrogen fertilization and its interactions with low-concentration SO_2 significantly increased herbage yield. Sulfur and nitrogen contents, as well as N:S ratios in the dominant species were altered by the treatments.

INTRODUCTION

In a previous paper we reported both positive and negative impacts of SO_2 on western wheatgrass (*Agropyron smithii* Rydb.) (Heitschmidt *et al.*, 1978). The positive response was an increase in the average number of leaves per plant as a result of SO_2 exposure. That plants can utilize SO_2 as a sulfur source is widely recognized for plants growing in sulfur-deficient soils (Faller, 1971; Cowling *et al.*, 1973; Cowling and Lockyer, 1978). Although the soils in our study area are not regarded as sulfur deficient (Wight, 1976), N:S ratios ranging from 15 to 30 were measured on the control plots early in the growing season.

The negative effect of SO_2 on western wheatgrass was an increase in leaf senescence. Other investigators have observed increased leaf senescence as a result of exposing plants to SO_2 (Bleasdale, 1973; Bell and Clough, 1973), which has been explained as the result of toxic concentrations of sulfate (Malhotra and Hocking, 1976).

This paper reports results of an experiment designed to test hypotheses about both sulfur deficiency and sulfur toxicity. This was accomplished by manipulating the soil sulfur and nitrogen concentrations on small plots within the large plots exposed to SO_2 treatments (Heitschmidt *et al.*, 1978).

Experimental Design

A split-plot design with replication was used. The main plots were four SO_2 treatments (Heitschmidt *et al.*, 1978). The split plots were nitrogen, sulfur, nitrogen plus sulfur, and glucose. The objective of the nitrogen and sulfur fertilizer treatments was to provide 2 percent nitrogen, 0.2 percent sulfur, or both in live tissue at peak standing crop. To accomplish this, we applied the equivalent of 150 kg ha^{-1} nitrogen (ammonium nitrate) and/or 15 kg ha^{-1} sulfur (magnesium sulfate) on 15 April. The glucose treatment consisted of applying the equivalent of 850 kg ha^{-1} of *D*-glucose. The objective of this was to reduce the availability of nitrogen to plants by increasing microbial populations (Behera and Wagner, 1974).

The nitrogen and sulfur treatments were replicated twice within each SO_2 treatment. Each treatment plot was 1 × 2 m. The glucose treatment was replicated twice in the control and high-SO_2 treatment only.

METHODS

Standing crop biomass above and belowground was measured at the end of the growing season by the harvest method. Aboveground biomass was measured by clipping one 0.25-m^2 quadrat in each of the fertilizer or glucose treatment plots. Plants were clipped at the soil surface and separated by species. Belowground biomass was measured by removing three soil cores, 7.5 cm diameter × 10 cm deep, from each harvested quadrat. Crowns, rhizomes, and roots were separated from the mineral soil by the method of Lauenroth and Whitman (1971). All plant material was oven dried at 60°C to a constant weight before weighing.

Western wheatgrass was analyzed for total sulfur with a Leco Induction Furnace (Laboratory Equipment Corp., St. Joseph, MO, USA), and total nitrogen (Kjeldahl N) by procedures in A.O.A.C. (1965).

Statistical analyses of the results were by analyses of variance with Tukey's Q values used to compare means (Snedecor and Cochran, 1967). Because the sulfur, nitrogen, and sulfur plus nitrogen treatments were applied across all SO_2 treatments and the glucose treatment was applied only to the control and high concentration treatment, two separate analyses were performed. The first analyses included four SO_2 and four sulfur/nitrogen treatments. The second considered two SO_2 treatments and five sulfur/nitrogen treatments.

RESULTS

Aboveground yield was significantly altered only by the addition of nitrogen fertilizer (Table 15.1, Figure 15.1). Yields of plots receiving nitrogen were significantly greater than those of plots not receiving nitrogen, regardless of SO_2 concentration. Combinations of nitrogen and SO_2 produced a significant interaction, the nitrogen/low SO_2 treatment producing a significant increase in yield over all other nitrogen/SO_2 combinations. Increased SO_2 concentrations above the low treatment caused significant decreases in yield compared with the low treatment but no distinguishable differences from the control. Yields for treatments not receiving nitrogen fertilizer were similar, regardless of SO_2 concentration (Table 15.1, Figure 15.1). Results from analysis of the glucose treatment produced similar conclusions. The reductions in yield observed on the glucose treatments were not statistically significant.

TABLE 15.1. TOTAL ABOVEGROUND YIELD* ON 15 AUGUST (g · m^{-2})

	Fertilizer Treatment				
SO_2 Treatment	Control	Sulfur	Nitrogen	Nitrogen plus sulfur	Glucose
Control	177 ± 48	120 ± 44	240 ± 64	368 ± 4	100 ± 24
Low	156 ± 52	152 ± 8	448 ± 32	480 ± 40	-
Medium	104 ± 8	120 ± 24	352 ± 28	328 ± 32	-
High	124 ± 24	140 ± 32	272 ± 72	260 ± 4	72 ± 4

* ± 1 S.E.

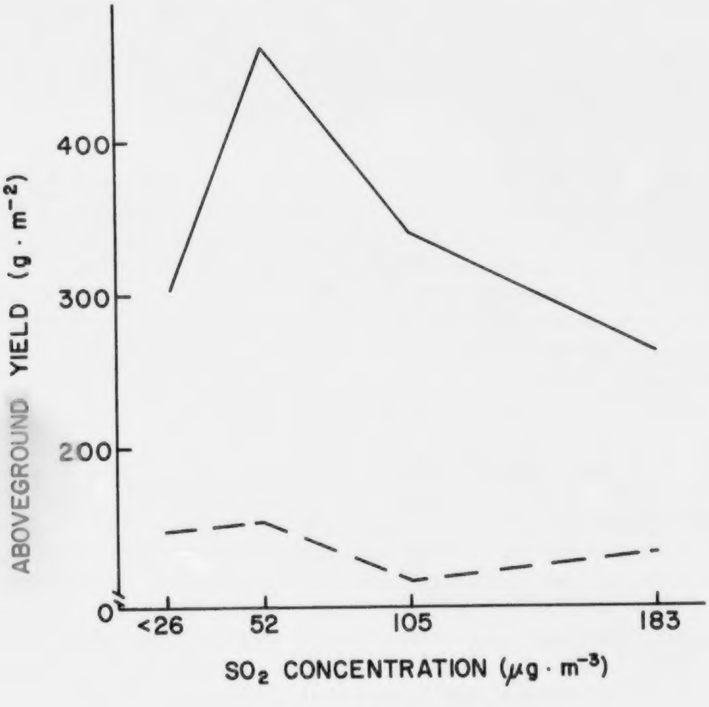

Figure 15.1. Aboveground yield as a function of SO_2 treatment and nitrogen fertilization. (Solid line is with added nitrogen, dashed line is without added nitrogen.)

Belowground standing crop was not significantly changed by any of the treatments. This result was probably as much the result of our inability to distinguish live roots as of the reduced responsiveness of belowground organs to perturbations compared with aboveground organs (Dodd and Lauenroth, 1979).

Sulfur concentrations in the aboveground biomass of western wheatgrass (Figure 15.2) were significantly related to the interaction of sample date, SO_2 treatment, and nitrogen treatment. Under nominal nitrogen conditions sulfur concentrations of plants growing on the medium and high concentrations treatments increased with both treatment and time (Figure 15.2a). Addition of mineral nitrogen reversed this trend, resulting in significant decreases in sulfur content of western wheatgrass between June and mid-August (Figure 15.2b). Treatment with glucose had no effect on the sulfur content of western wheatgrass under control conditions (Figure 15.2c,d) but significantly increased sulfur content on the July and August sample dates in the high SO_2 treatment, compared with other amendments.

Nitrogen concentrations were significantly related to interactions of sample date and nitrogen treatment, as one would expect (Figure 15.3). June nitrogen concentrations were significantly increased by N fertilization. This effect diminished throughout the growing season. Mid-August N contents were the same, regardless of treatment. Glucose had no effect on nitrogen content.

Significant changes in N:S ratios were largely related to time (Figure 15.4). SO_2 treatment decreased N:S ratios as a result of its influence on S concentration. Nitrogen additions significantly increased N:S ratios on the final sample date (Figure 15.4b), and S fertilization significantly decreased N:S ratios early in the growing season (Figure 15.4c). Additions of glucose had no influence upon N:S ratios.

DISCUSSION

Sulfur dioxide is widely recognized as a phytotoxic air pollutant (Ziegler, 1975) but is less widely understood as a potential source of an important plant nutrient (Cowling and Lockyer, 1978). Because of its unique position among phytotoxic gases as both a nutrient and toxic agent, any assessment of the responses of plants to sulfur dioxide must consider both positive and negative impacts. In a very general sense the relationship between sulfur dioxide concentration and a measure of plant performance is expected to be non-linear with a single optimum. This is the subsidy-stress gradient described by Odum *et al.* (1979). For example, a plant grown in a sulfur-free rooting medium and exposed to a range of concentrations of sulfur dioxide may be expected to achieve a maximum growth rate at a sulfur dioxide concentration supplying sulfur at a rate very close to the plant's demand. At higher concentrations toxicity may occur, and at lower concentrations sulfur deficiency will limit growth. The situation for a plant growing as an individual in a community is complicated by the large number of variables that can be expected to modify its response to sulfur dioxide.

The responses illustrated in Figure 15.1 clearly demonstrate a gradient in responses of aboveground yield of a grassland to a range of SO_2 treatments. The important determinant of this response is the presence of adequate mineral

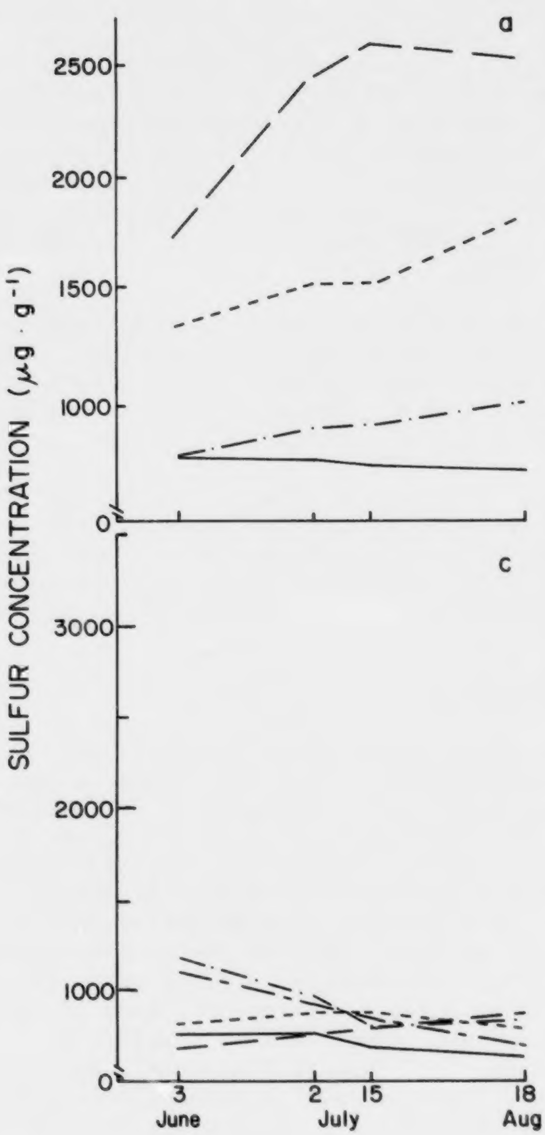

Figure 15.2. Sulfur content of western wheatgrass: (a) without additions of N, (b) with additions of N (control —— , low -·- , medium --- , high — —), (c) in the absence of SO_2, (d) under high concentration SO_2 (control —— , sulfur added --- , nitrogen added —— - —— , nitrogen and sulfur added — · — , glucose — —).

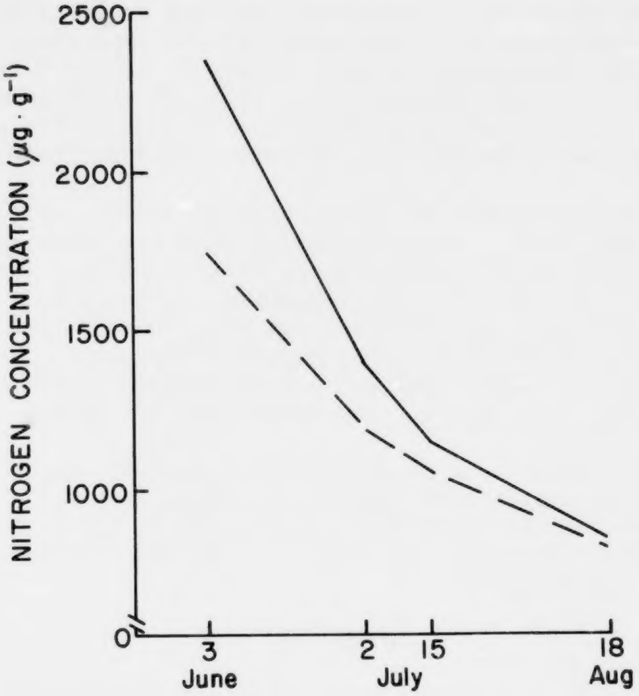

Figure 15.3. Nitrogen content of western wheatgrass over the growing season. (Solid line is with added nitrogen, dashed line without added nitrogen)

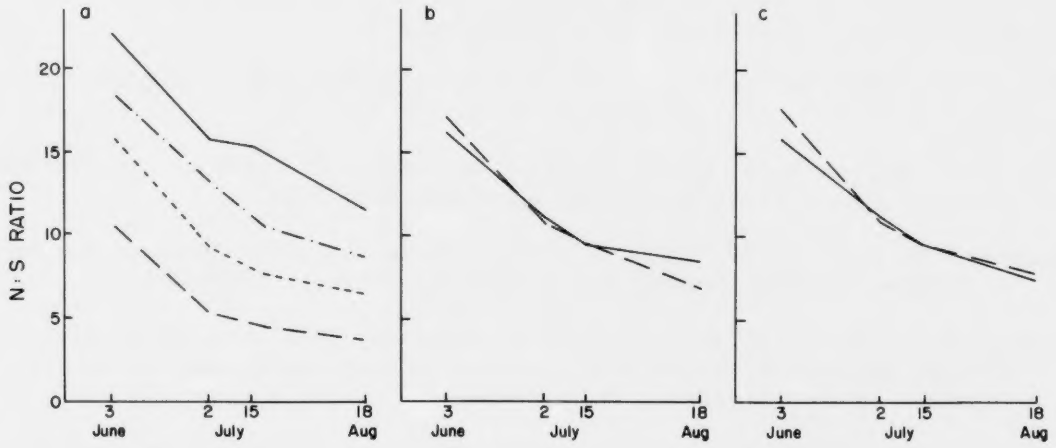

Figure 15.4. Nitrogen to sulfur ratios in western wheatgrass: (a) related to SO_2 treatment (control —— , low -·- , medium --- , high — —), (b) with (solid line) and without (dashed line) the addition of N, (c) with (solid line) and without (dashed line) the addition of S.

nitrogen. Noggle and Jones (1979) suggested that the presence of SO_2 in the atmosphere in the southeastern U.S. has prevented the expression of sulfur-deficiency symptoms in agricultural crops to which N, P, and K fertilizers are commonly applied. Our results indicate that, although their interpretation may be correct, the response for grasslands is sensitive to SO_2 concentration and is only positive over a narrow range of concentrations (Figure 15.1).

Fertilization studies conducted in the Northern Great Plains with the objective of increasing range forage production have not shown responses to sulfur fertilization with or without nitrogen (Wight, 1976). Our results document significant yield response to atmospheric sulfur inputs and large but non-significant responses to sulfur fertilizer when applied with nitrogen (Table 15.1). Evidence from N:S ratios suggests that, during rapid growth, sulfur cannot be taken up rapidly enough to meet demands. Apparently, atmospheric sulfur inputs as SO_2 can compensate for slow uptake.

Wight and Black (1979) reported average increased forage yields of 43 percent over 8 years of experimentation with the addition of 112 kg ha^{-1} nitrogen. Yield increases for individual years ranged from less than 25 percent to more than 100 percent. We found a 35 percent increase in yield as a result of N fertilization alone.

CONCLUSIONS

Because sulfur is an important mineral nutrient in grasslands its potential actions with nitrogen must be considered in assessing impacts of atmospheric sulfur imputs.

REFERENCES

A.O.A.C. 1965. Official Methods of Analysis, 10th ed. Association of Official Agricultural Chemists, Washington, D.C.

Behera, B., and G. H. Wagner. 1974. Microbial Growth Rate in Glucose Amended Soil. Soil Sci. Soc. Am. Proc., 38:591-594.

Bell, J. N. B., and W. S. Clough. 1973. Depression of Yield in Ryegrass Exposed to Sulfur Dioxide. Nature (London), 241:47-49.

Bleasdale, J. K. A. 1973. Effects of Coal Smoke Pollution Gases on the Growth of Ryegrass (*Lolium perenne* L.). Environ. Pollut., 5:275-285.

Cowling, D. W., L. H. P. Jones, and D. R. Lockyer. 1973. Increased Yield Through Correction of Sulfur Deficiency in Ryegrass Exposed to Sulfur Dioxide. Nature (London), 243:479-480.

Cowling, D. W., and D. R. Lockyer. 1978. Effect of SO_2 on *Lolium perenne* Grown at Different Levels of Sulfur and Nitrogen Nutrition. J. Exp. Bot., 29:257-266.

Dodd, J. L., and W. K. Lauenroth. 1979. Analysis of the Response of a Grassland Ecosystem to Stress, pp. 43-58. In: N. R. French, ed. Perspectives in Grassland Ecology. Springer-Verlag, New York, 204 pp.

Faller, N. 1971. Plant Nutrient Sulfur-SO_2 *vs.* SO_4. Sulfur Inst. J., 7:5-6.

Heitschmidt, R. K., W. K. Lauenroth, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulfur Dioxide on Western Wheatgrass in a Southestern Montana Grassland. J. Appl. Ecol., 14:859-868.

Lauenroth, W. K., and W. C. Whitman. 1971. A Rapid Method for Washing Roots. J. Range Manage., 24:308-309.

Malhotra, S. S., and D. Hocking. 1976. Biochemical and Cytological Effects of Sulfur Dioxide on Plant Metabolism. New Phytol, 76:227-237.

Noggle, J. C., and H. C. Jones. 1979. Accumulation of Atmospheric Sulfur by Plants and Sulfur-supplying Capacity of Soils. Interagency Energy/Environment R & D Program Rep. TVA, EPA EPA-600/7-79-109. 37 pp.

Odum, E. P., J. T. Finn, and E. H. Franz. 1979. Perturbation Theory and the Subsidy-stress Gradient. Bio. Sci., 29:349-352.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State Univ. Press, Ames.

Wight, J. R. 1976. Range Fertilization in the Northern Great Plains. J. Range Manage., 29:180-185.

Wight, J. R., and A.L. Black. 1979. Range Fertilization: Plant Response and Water Use. J. Range Manage., 32:345-49.

Ziegler, I. 1975. The Effect of SO_2 Pollution on Plant Metabolism. Residue Rev., 56:79-105.

SECTION 16

EFFECTS OF LOW-LEVEL SO_2 FUMIGATION ON DECOMPOSITION OF WESTERN WHEATGRASS LITTER IN A MIXED-GRASS PRAIRIE

J. L. Dodd and W. K. Lauenroth

ABSTRACT

Litter bags were used to determine the effects of SO_2 and substrate-S on field decomposition rates of western wheatgrass leaves. In our study atmospheric SO_2 decreased seasonal decomposition rates by about 10% while increased S content of substrate (.098% vs .059% in control) had no measurable effect. The observed inhibition is probably due to reduced pH and/or accumulation of toxic derivatives of SO_2 in the microenvironment of decay organisms on the decaying leaf surfaces.

INTRODUCTION

Most aboveground net primary production in native grasslands passes through the decomposition process at ground level. In ungrazed grasslands nearly all the aboveground production eventually decays on or near the surface, while in most properly grazed grasslands half or more of the aboveground production is decayed by surface processes. Volatile products from this process are lost from the system. Other inorganic and organic products become directly involved in subsurface nutrient cycling and energy transformation processes that are critical in maintaining ecosystem stability and primary productivity. Strojan (1978) reported that decomposition rates for tree leaves were greatly reduced near a zinc smelter in Pennsylvania. He attributed this to less biological activity in the leaf litter brought about by increased litter concentrations of Cd, Cu, Fe, Pb, Zn, and S originating from the smelting process.

The purpose of this study was to determine the effects of atmospheric SO_2 on decomposition rates of leaves of western wheatgrass (*Agropyron smithii*), the dominant native grass of the Northern Great Plains, and whether the rates were altered by sulfur content of the leaf tissue.

MATERIALS AND METHODS

In March of 1978, before initiation of fumigation on the treatment plots, standing dead western wheatgrass leaves were collected from one location in the control and high-SO_2 treatment plots. The leaf tissue collected was grown in 1977 under control and high SO_2 treatment conditions. Each source of leaf material was oven dried (24 h @ 60°C) and subsampled for determinations of total S (Leco Induction Furnace, Laboratory Equipment Corp., St. Joseph, Missouri) and for ash content (A.O.A.C, 1965).

A sample of intact leaves weighing approximately 250 mg (ash-free), were weighed and placed in litterbags (30 x 15 cm) made of 1-mm opening nylon mesh. One-hundred litterbags of each of three series of samples were prepared. Series A(A), material grown on the control plot, and series A(D), material grown on the high-SO_2 treatment plot, were placed in the control plot. Series D(D), material grown on the high-SO_2 treatment plot, was placed in the high-SO_2 treatment. All litterbags were placed in the field on April 15, 1978. Litterbags were laid flat on the ground between clumps of grass. Each bag was attached to the soil surface with nails to assure close contact of the contents with the soil.

Twenty litterbags from each series were retrieved on each of five dates (18 May, 16 June, 15 July, 15 August, and 16 September). Contaminating debris was removed and the contents of each bag were oven dried, weighed, ashed, and reweighed to determine organic weight. Decomposition rates for the three test series were expressed as percent of organic weight remaining since April 15. Daily rates of loss ($mg \cdot g^{-1} \cdot d^{-1}$) were computed for each ≅ 30-day interval of the season from the average percent of material remaining at each date. Estimates of variance for this statistic could not be computed, because organic weights of the individual samples at the beginning of each interval were not measurable (except for the first interval).

Precipitation was measured on the control and SO_2-treated test areas from May through August. Differences between test plots were not significant and averaged 263, 57, 28, and 13 mm for May, June, July, and August, respectively.

RESULTS AND DISCUSSION

The March 1978 sulfur contents ($\bar{X} \pm SE$) of dead western wheatgrass leaves grown on the control and SO_2-treated plots in 1977 were 0.059 ± 0.0035% and .098 ± .0015%, respectively.

Significant differences in rates of organic weight losses were found between sample material decayed on the control plot and on the SO_2-treated plot (Table 16.1). By the end of the 154-d test period, 76.3% of the leaf material remained in the SO_2-treated samples, compared with 66.5% in the control samples. Differences in organic weight loss due to substrate sulfur contents at the beginning of decay period were not detected.

Average daily loss rates were greatest between 18 May and 15 July and least between 15 August and 16 September (Table 16.2). The daily loss rates for leaf material decayed in the SO_2 treatment ranged from 56 to 88% of the rates for materials decayed in the control plot.

TABLE 16.1. PERCENT* OF *AGROPYRON SMITHII* LEAF LITTER WEIGHT (ASH-FREE, DRY WEIGHT) REMAINING SINCE APRIL 15, 1978

	A(A)[†]	A(D)[‡]	D(D)[§]
18 May	91.9 ± .7	94.2 ± .4	95.4 ± .6
16 June	83.4 ± .9	84.8 ± .5	89.3 ± .8
15 July	75.4 ± .9	77.7 ± 1.4	83.7 ± 1.0
15 August	70.4 ± 1.3	72.1 ± 1.7	80.4 ± 1.3
16 September	66.5 ± 1.3	67.2 ± 1.6	76.3 ± 1.2

* $\bar{X}$ ± SE.

[†] A(A) = Tissue grown and decomposed on control.

[‡] A(D) = Tissue grown on high SO_2 concentration plots and decomposed on control.

[§] D(D) = Tissue grown on and decomposed on high-SO_2 concentration plots.

TABLE 16.2. RATES OF LOSS* OF *AGROPYRON SMITHII* FOR FIVE INTERVALS DURING 1978

	A(A)	A(D)	D(D)
15 April - 18 May	2.5	1.8	1.4
18 May - 16 June	3.1	3.3	2.1
16 June - 15 July	3.2	2.8	2.1
15 July - 15 August	2.1	2.3	1.2
15 August - 16 September	1.7	2.1	1.5

* $mg \cdot g^{-1} \cdot d^{-1}$.

CONCLUSIONS

Our results indicate that low levels of atmospheric SO_2 inhibits decomposition rates of western wheatgrass during, at least, the first year in the decomposition cycle. The results also suggest that the inhibition is least during the latter part of the season, when surface conditions are quite dry.

The decomposition rate probably slows because of reductions in pH and/or accumulations of toxic SO_2 derivatives (SO_3, SO_4) in the microenvironment of decay organisms on the decaying leaf surfaces. These changes likely result from sorption of SO_2. Since both sorption of SO_2 on the surfaces and decomposition rates would normally be greatest under wet conditions, the probability of inhibition would be greatest early in the season.

REFERENCES

A.O.A.C. 1965. Official Methods of Analysis. 10th edn. Assoc. of Official Agr. Chemists, Washington, D.C.

Strojan, C. L. 1978. Forest Leaf Litter Decomposition in the Vicinity of a Zinc Smelter. Oecologia, (Berl.) 32:203-212.

SECTION 17

PLANT COMMUNITY STRUCTURE ON ZAPS

J. E. Taylor, W. C. Leininger, and M. W. Hoard

ABSTRACT

Canopy coverage, species diversity, frequency, and western wheatgrass density were sampled periodically during the 1978 growing season in an attempt to assess the effects of sulfur dioxide fumigation on native rangeland vegetation. The two study plots (ZAPS I and ZAPS II) have been fumigated during the growing seasons since 1975 and 1976, respectively. Differences in vegetational cover are associated with inherent inter- and intra-plot variation. Other differences are demonstrably due to sulfur dioxide stress. Total living vegetation, total graminoids, and western wheatgrass show decreasing cover with increased SO_2 rates. Lichen cover likewise has decreased significantly. Litter cover has increased on all treatments for the first three years, probably due to grazing deferment. ZAPS I shows a significant litter reduction on the D plot the fourth year. Neither diversity nor evenness are consistent indicators of stress, although some depression has been observed. Species richness was decreased with fumigation when some species were lost and not replaced in the composition. Western wheatgrass densities on ZAPS I are significantly lowered by fumigation.

INTRODUCTION

The objective of this research is to determine whether low-level SO_2 concentration causes changes in vegetation on native rangeland. This question is being approached through studies of canopy coverage, species diversity, species frequency, and western wheatgrass (*Agropyron smithii*) density.

MATERIALS AND METHODS

Methodological details are presented in Section 4. The following discussion is restricted to modification and additions to those procedures.

Plant Community Analysis

Canopy Coverage

Coverage data were obtained following the method of Daubenmire (1959). Data were collected on the ZAPS sites at four times during the 1978 growing season: 3-5 June, 16-19 June, 14-15 July, and 9-11 September. These dates approximated the stages of early growth, peak of cool-season green, peak of warm season green, and summer dormancy, respectively.

Each of the ZAPS plots was subdivided for sampling, with equal observations collected from the northern and sourthern halves. These were handled as "replicates" in the statistical analyses, although they were not randomly designated.

No samples were taken in microtopographic depressions (run-in moisture areas) nor in areas less than one meter from a SO_2 delivery pipe.

To select plant community components for detailed analysis, a preliminary data review was accomplished. Several functional groups (perennials and annuals; grasses, forbs, and shrubs; dominant species) were evaluated as to their responses to treatment. Total living vegetation, total graminoids, western wheatgrass, lichens, and litter were found to show significant responses. Each of these categories was subjected to an analysis of variance. Significant treatments were examined with Duncan's multiple range test. These analyses were made for all treatment years.

Diversity

Diversity, evenness, and species richness were calculated at every sampling date on every plot. The Shannon-Weaver function (H') was used for diversity (Shannon Weaver, 1949). Evenness (equitability) was calculated with Pielou's Index (J') (Pielou, 1969). Species richness was taken as the numerical sum of plant species present in each five-frame group.

Frequency

Frequency data by species were extracted from the plot data used in cover analysis. These were grouped into five equal frequency classes. The number of species in each frequency class was plotted in an attempt to further characterize the evenness component of diversity. Number rather than percent data were used so that species richness also was accounted for. June, July, and September data from 1975 and 1978 were compared on ZAPS I only.

Western Wheatgrass Density

At the same time that cover estimates were being made on the 2 x 5 dm sample plots, numbers of western wheatgrass plants were counted on ZAPS I only. Plant units as described in Section 4 were used.

Photographic Monitoring

Ground Level Photography

Photoplots were recorded twice during 1978 using color film. Detailed vegetational charts were developed for each plot. Procedures are explained more fully in Section 4. New photoplots using both color and color infrared film were recorded on the fertilizer study plots being conducted by Colorado State University.

Aerial Photography

The aerial photographic activity conducted on the ZAPS sites is described in Section 22.

RESULTS AND DISCUSSION

Plant Community Analysis

In the following discussion, ZAPS I and II are considered separate studies rather than true replicates. They were established in different years, received different intensities of grazing pressure before fencing, are located on different sites, support different plant communities, and have received different patterns of precipitation. Also, SO_2 distribution characteristics are different because of design modifications in the ZAP system.

Further, the D plot on ZAPS II is not comparable to the other plots. It falls on an area with numerous microdepressions of dendritic rill form. This pattern was inconspicuous in the first years of study because pre-treatment grazing had reduced the plot aspect to a uniformly short sod. Thus, no sample stratification was employed. With deferment, it soon appeared that very different plant communities occupied the depressions and the intervening sites. Therefore, beginning with the 1978 samples, the microdepressions were avoided in sampling. For this reason, 1978 D plot data are not comparable to either earlier D plot data or to data from the other treatment plots. For one important example, western wheatgrass is less abundant in the microdepressions. This species is the dominant species and major contributor to both graminoid and total vegetation categories. Therefore, the D plot cover data were excluded from the statistical analyses of these groups.

Canopy Cover

Figure 17.1 shows canopy coverage by plant type for ZAPS I in 1978. Canopy coverage can exceed 100% due to overlapping crowns of different species. The seasonal differences in total vegetation were primarily due to normal phenologic patterns of vegetational development and to the loss of annuals at the later sampling dates. The ratio of forbs to grasses did not change much during the year. Lichens decreased with SO_2 fumigation. Shrubs were present in low amounts, and no trends in their cover were noted. Observation suggested that their distribution was largely a reflection of pretreatment patterns. Mosses varied with season, with no correlation between moss cover and SO_2.

The overall trends on ZAPS II were similar to those on ZAPS I (Figure 17.2). The period of maximum plant growth appeared earlier, reflecting the drier and warmer nature of the ZAPS II site. More ephemeral forbs were present at the first sampling date. The trend toward decreased coverage as the season progresses is seen clearly. Graminoid coverage was comparable on all treatments. Forbs became relatively less conspicuous with the advancing season, and may also have shown a reduction due to fumigation. Dandelion (*Taraxacum officinale*) was the predominant forb species in the early season.

Table 17.1 summarizes the statistical significance of treatment means as determined by analysis of variance. Where significant means were encountered, Duncan's multiple range tests were made.

TABLE 17.1. SIGNIFICANCE OF TREATMENT MEANS, ZAPS I AND II, 1975-78

Components of Vegetation	ZAPS I				ZAPS II		
	1975	76	77	78	1976	77	78
Western wheatgrass	*	NS	**	**	**	**	**
Graminoids	**	*	**	**	NS	**	**
Lichens	*	*	**	*	*	**	**
Total living vegetation	**	*	*	**	NS	NS	*
Litter	NS	*	*	*	NS	NS	**

NS = not significant
* = significant (P=.05)
** = highly significant (P=0.01)

Total living vegetational cover is shown in Figures 17.3 and 17.4. Initially the D plot on ZAPS I had the highest total cover, but by the second year the control plot had increased about 25%, while the fumigated plots remained at about 1975 levels. This may be a negative treatment response whereby SO_2 is inhibiting vigor enhancement from grazing deferment. The tendency for increased cover on the control relative to fumigation treatments continues in subsequent years.

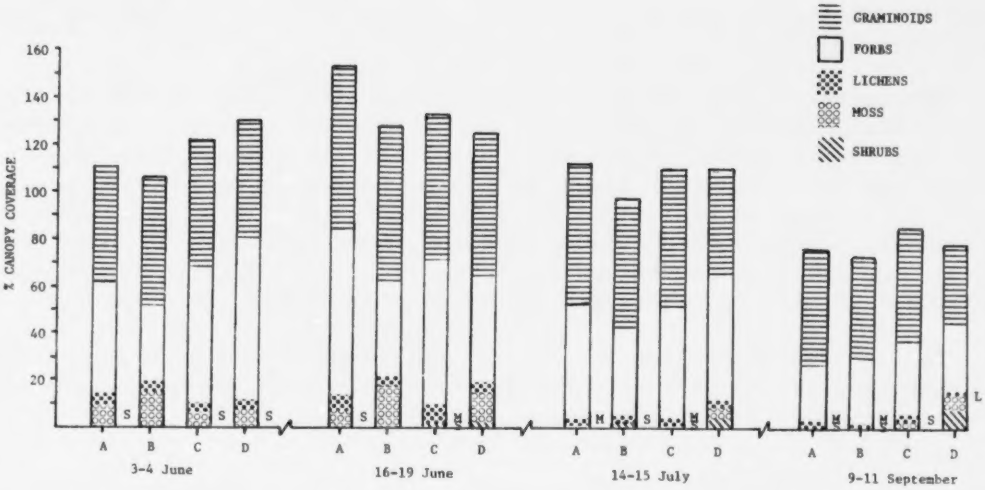

Figure 17.1. Percent canopy coverage for 4 sample dates, ZAPS I, 1978.

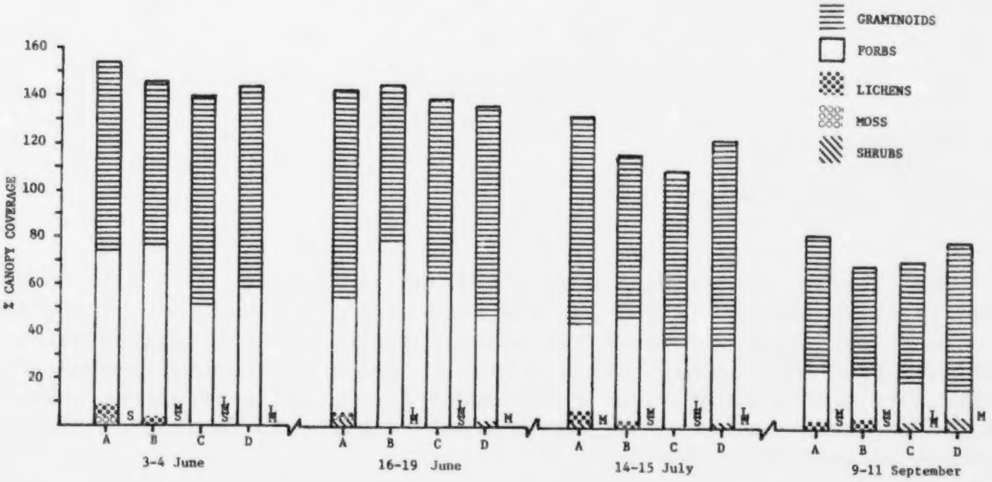

Figure 17.2. Percent canopy coverage for 4 sample dates, ZAPS II, 1978.

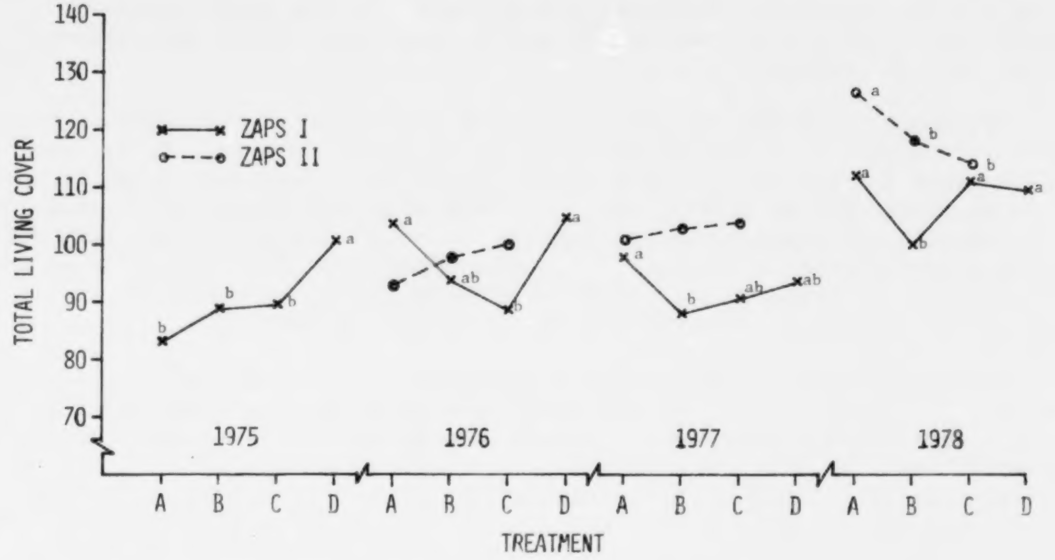

Figure 17.3. Total living vegetational coverage on ZAPS I and II, 1975-1978. Means within years and ZAPS not followed by the same letter are significantly different at the .05 level.

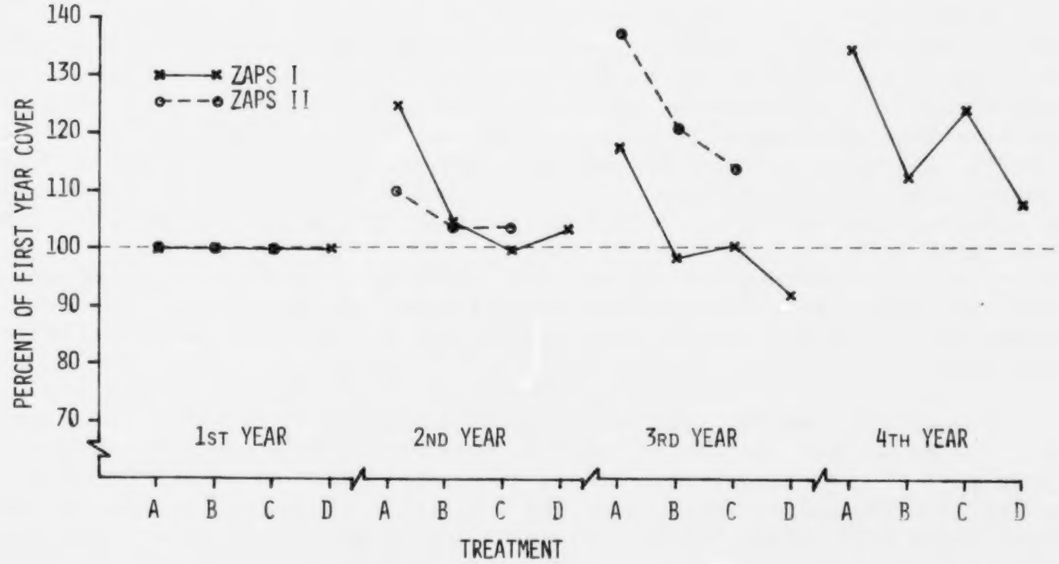

Figure 17.4. Total living cover expressed as a percentage of first year cover, ZAPS I and II. (Years refer to longevity of SO_2 fumigation.)

Total living vegetational cover on ZAPS II showed similar trends, with less variable treatment responses within years. By the third fumigation season the B and C plots had significantly less total living vegetational cover than the control.

The graminoids are the plant component of highest significance to rangeland utilization by livestock and wildlife in the study area. Their cover is illustrated in Figures 17.5 and 17.6. There is a clear depression in cover with increased SO_2 on ZAPS I. By the second year, all levels of SO_2 fumigation showed less graminoid cover than the control. For the D plot this difference was statistically significant every year. By 1978 a significant negative linear relationship was observed between fumigation rates (Table 17.1) and graminoid cover. No clear trends are evident on ZAPS II.

Western wheatgrass is a large contributor to the graminoid trends Figures 17.7 and 17.8). In the first year of fumigation there was significantly more western wheatgrass on the D than on the A plot; B and C were intermediate. In 1976 there were no significant treatment effects, but there was a noticeable increase in coverage on the control. By 1978 the A plot cover was significantly greater than that of any of the fumigated plots. This was produced by concommitant increases in control plot cover and decreases in fumigated plot cover, especially at the higher rates. (Figure 17.8). On ZAPS II the C plot has had significantly less western wheatgrass cover than A and B every year. This disparity is increasing with time, primarily due to a reduction in C plot. This has potentially serious economic implications since this species is the predominant forage plant of the area, and indeed of much of the entire mixed prairie region.

Forbs are the most dynamic component of the vegetation system on the ZAPS sites. They are highly responsive to annual and seasonal differences in climate, and are usually the first group of plant species to change in composition with disturbance, drought, or other ecological factors. When they are considered as a group rather than as individual species, the situation is made more difficult because many are relatively short-lived or short-season plants. Thus, seasonal trends represent the responses of a succession of species, even when year-to-year composition does not vary. Even so, there is a tendency for a forb cover to increase less on the higher SO_2 plots, even under favorable growing conditions. For example, on ZAPS I, when compared with the control plot, forb values in 1978 were well below their relative values in 1975 on the D plot, even though 1978 was the more favorable moisture year.

Annual and biennial species increased dramatically in 1978. This seems more due to weather variability than to treatment. This group never is a large part of the composition, and is so variable that it is difficult to analyze statistically. Also, some are very early ephemeral species and complete their life cycles before fumigation begins each year, so the only SO_2 effects they would show would be from sulfur remaining in the soil from previous years' fumigation. Therefore, the use of these groups as biomonitors is limited.

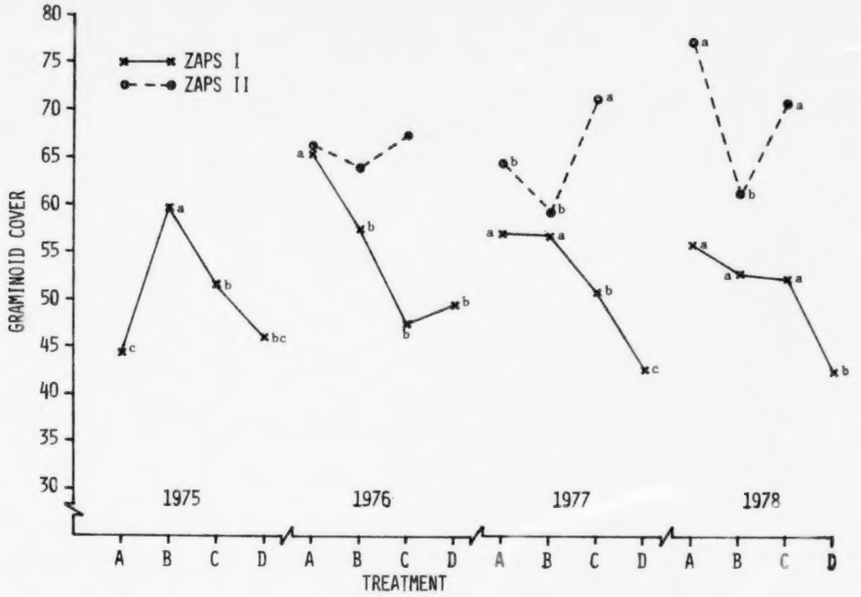

Figure 17.5. Graminoid cover on ZAPS I and II, 1975-1978. Means within years and ZAPS not followed by the same letter are significantly different at the .05 level.

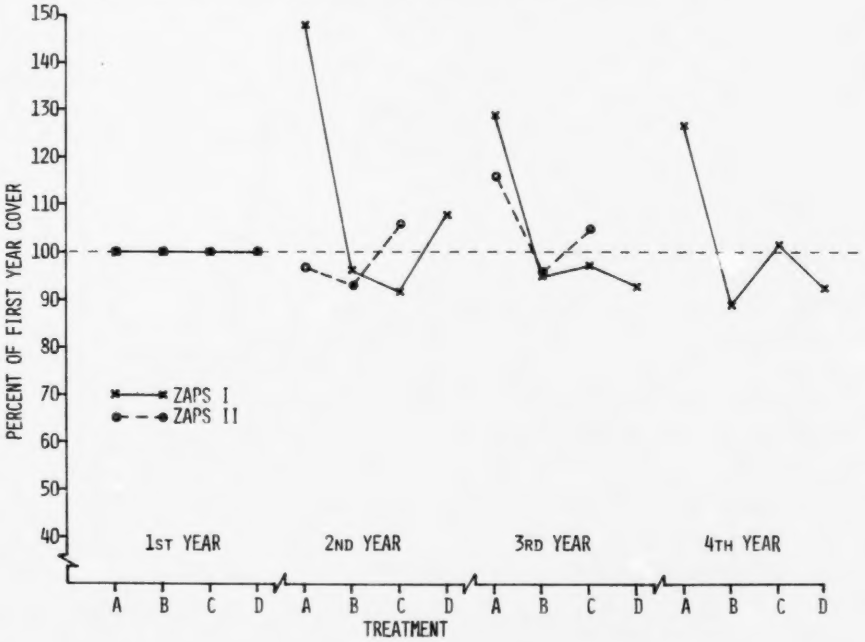

Figure 17.6. Graminoid cover expressed as a percentage of first year cover, ZAPS I and II. (Years refer to longevity of SO_2 fumigation.)

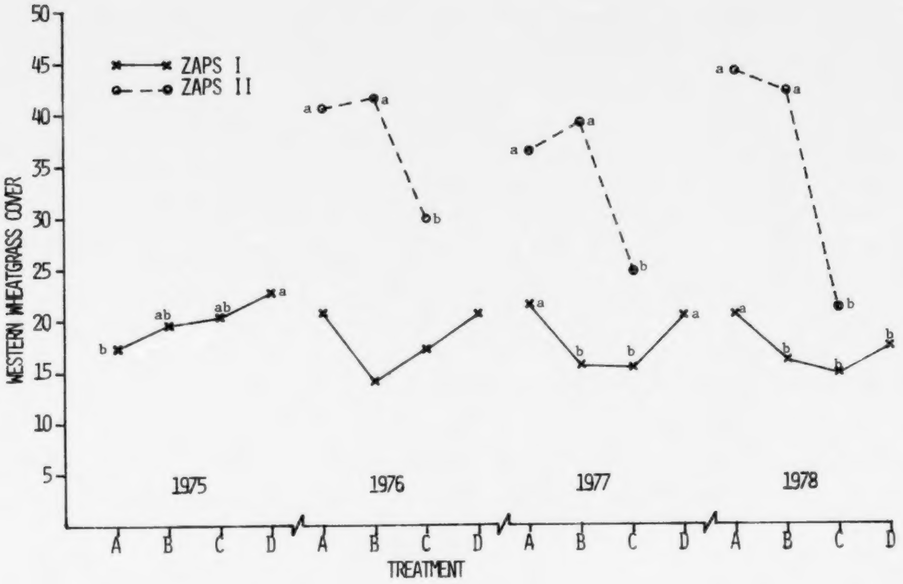

Figure 17.7. Western wheatgrass cover on ZAPS I and II, 1975-1978. Means within years and ZAPS not followed by the same letter are significantly different at the .05 level.

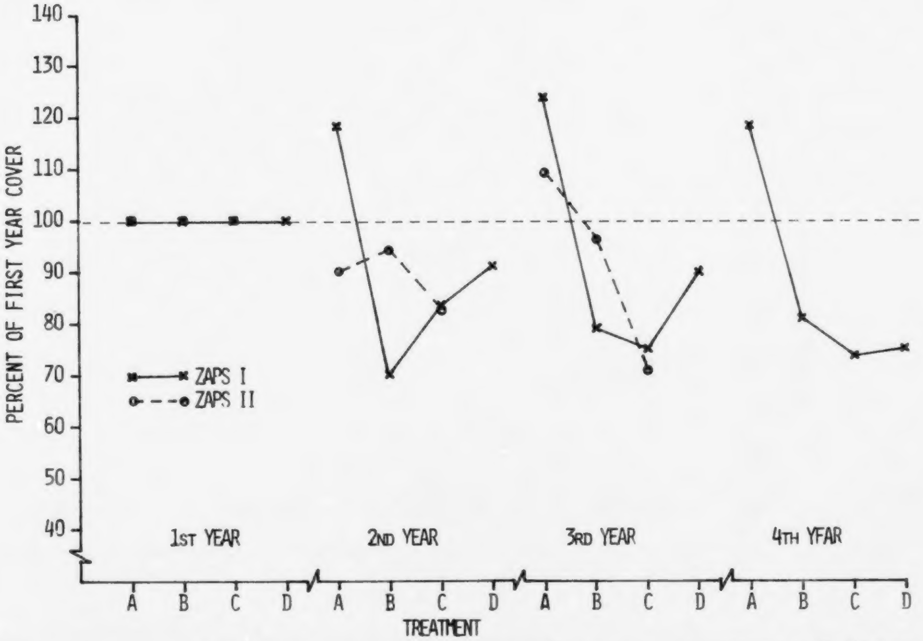

Figure 17.8. Western wheatgrass cover expressed as a percentage of first year cover, ZAPS I and II. (Years refer to longevity of SO_2 fumigation.)

One conspicuous phenomenon on both ZAPS sites is an accumulation of litter (Figures 17.9 and 17.10). This is a product of decreased rates of decomposition under sulfur dioxide fumigation (see Section 16) and grazing deferment. Even though initial litter cover differed between the ZAPS sites, both areas showed highly significant linear increases ($r = 0.88$ and $r = 0.94$ for ZAPS I and II, respectively) over the first three years of treatment (Figure 17.9). In the fourth year litter build-up on ZAPS I apparently has stabilized. Analysis of variance revealed no significant treatment differences for either ZAPS. By the third season of fumigation, both ZAPS showed significantly more litter cover on the D plots than on the controls. In the fourth year (attained only by ZAPS I), the opposite result was seen. The decreased litter on the D plot probably is a function of reduced vegetational production under fumigation.

In 1978 lichen reductions with SO_2 again were observed (Figures 17.11 and 17.12). This agrees with earlier findings (Eversman, 1978, 1979; Taylor and Leininger, 1979). Some yearly variations exist, but the trend clearly remains for lichen cover to decrease under fumigation. After the first season, the D plot has significantly less lichen cover than the A plot in every case. On ZAPS II there is a linear decrease. Pretreatment values on ZAPS I and their subsequent decreases are particularly striking.

This trend is shown more clearly when the lichen cover of 1978 is expressed as a percentage of the first year value (Figure 17.13). On ZAPS I all levels are less than those observed in 1975, with a down-trend associated with fumigation. The ZAPS II A plot lichen cover still is comparable with that of 1976, but all others are depressed, though not in as linear a fashion. It may be that since these plots have received one year less fumigation than ZAPS I, there has not been enough cumulative effect to stabilize the curve, or it may be due to the different growth conditions on the two ZAPS sites, as discussed earlier.

Another way of looking at lichen cover changes is shown in Figure 17.14. Here, each year's cover is expressed as a percentage of the cover observed in the first year of fumigation. In 1975, all ZAPS I plots were of course at 100% of that year's levels, yielding a horizontal curve. ZAPS II was not yet established. In 1976, lichen cover on ZAPS I was below the first year values on all plots. Except for the anomolous relative increase on the B plot, a substantial reduction was observed with SO_2 fumigation. ZAPS II was in its first year. In 1977, very strong depressions in lichen cover were observed on both sites. The response curves were very similar. In 1978, ZAPS II closely traced its curve of the previous year, but on ZAPS I, all plots including the A plot were well below first-year levels. It is not known whether the depression in control cover is due to cumulative low SO_2 exposures or to different growth conditions than the ZAPS II site experienced in 1978. Even so, the C and D plots continued to support less lichen cover than the A plot.

During the past two years, the biological significance of this lichen loss has been presumed to be as an early indication of ecosystem disruption which might foreshadow similar changes in more economically important species.

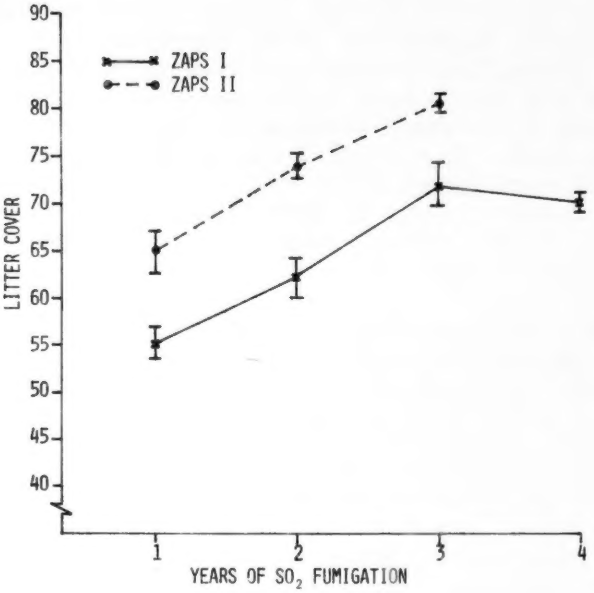

Figure 17.9. Litter cover (means and standard errors) for ZAPS I and II.

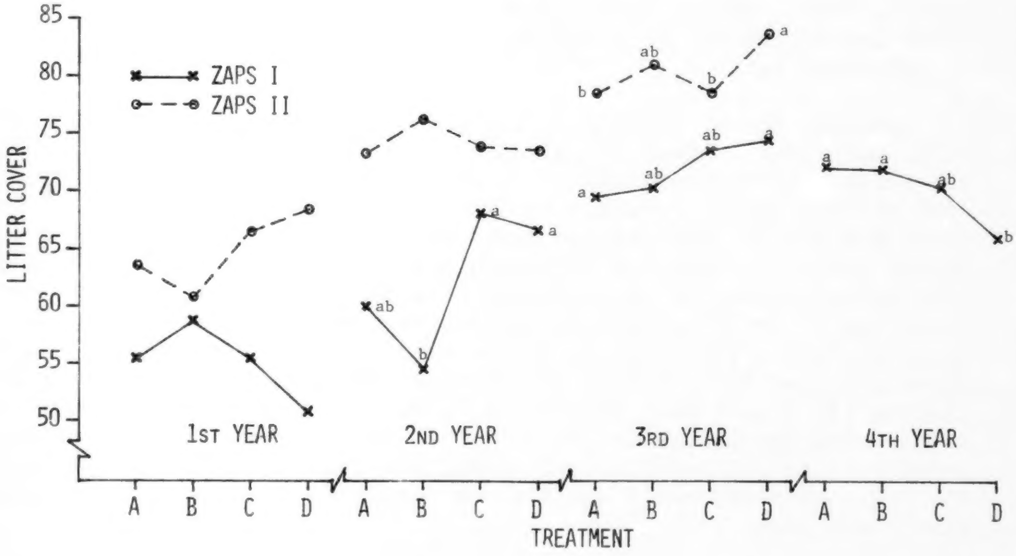

Figure 17.10. Litter cover on ZAPS I and II. First year on ZAPS I, 1975, and first year on ZAPS II, 1976. Means within years and ZAPS not followed by the same letter are significantly different at the .05 level.

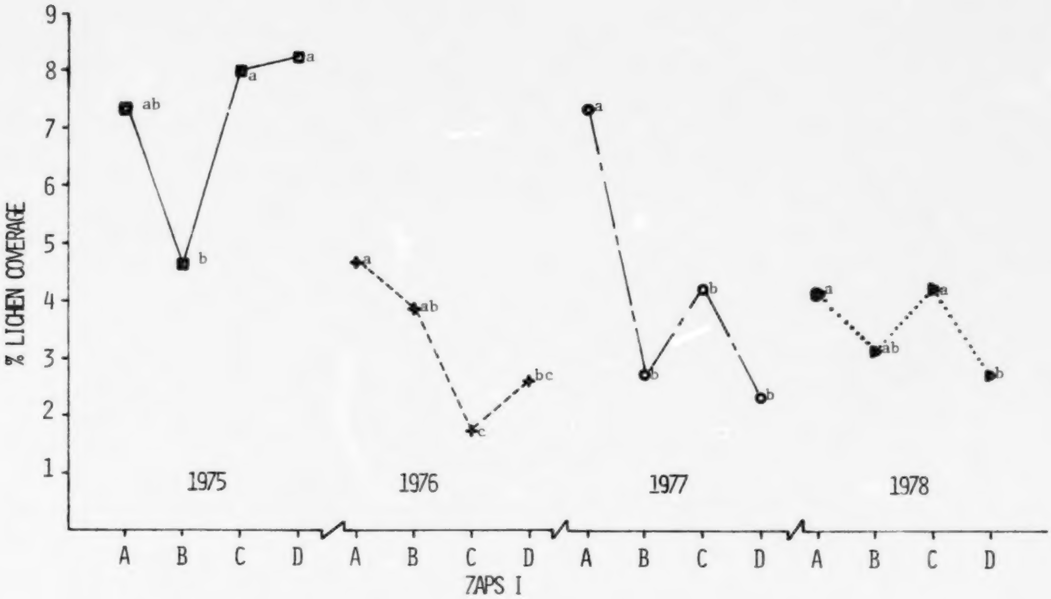

Figure 17.11. Mean lichen coverage for ZAPS I, 1975-1978. Means within years not followed by the same letter are significantly different at the .05 level.

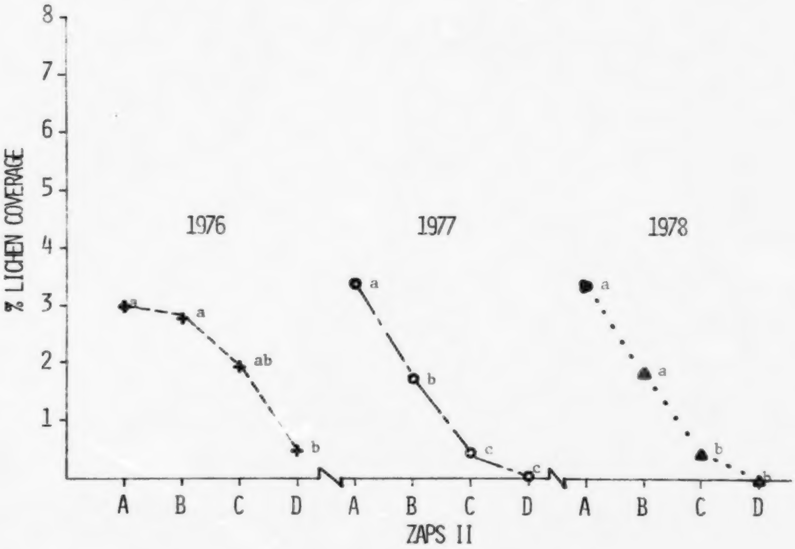

Figure 17.12. Mean lichen coverage for ZAPS II, 1976-1978. Means within years not followed by the same letter are significantly different at the .05 level.

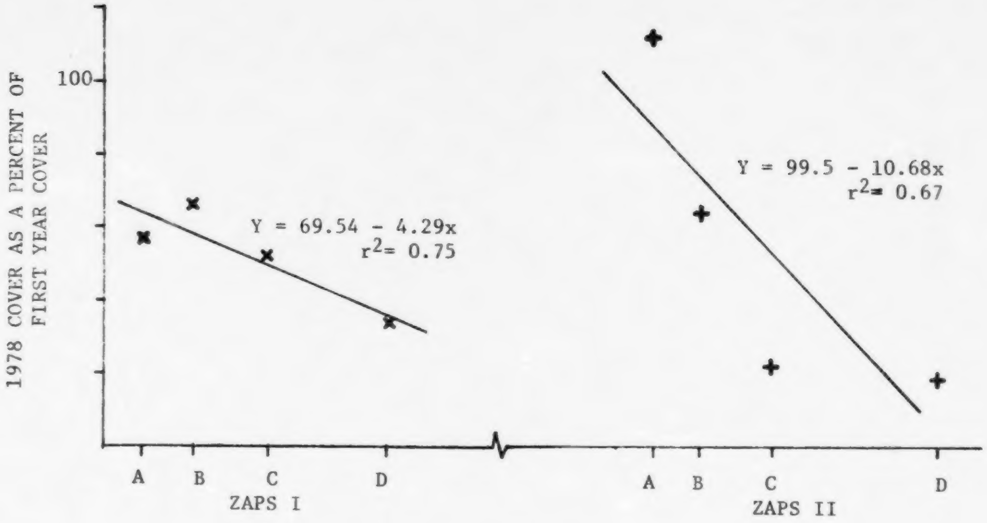

Figure 17.13. Lichen cover expressed as a percentage of first year cover, ZAPS I and II, 1978. SO_2 levels are seasonal geometric means observed in the field (see Section).

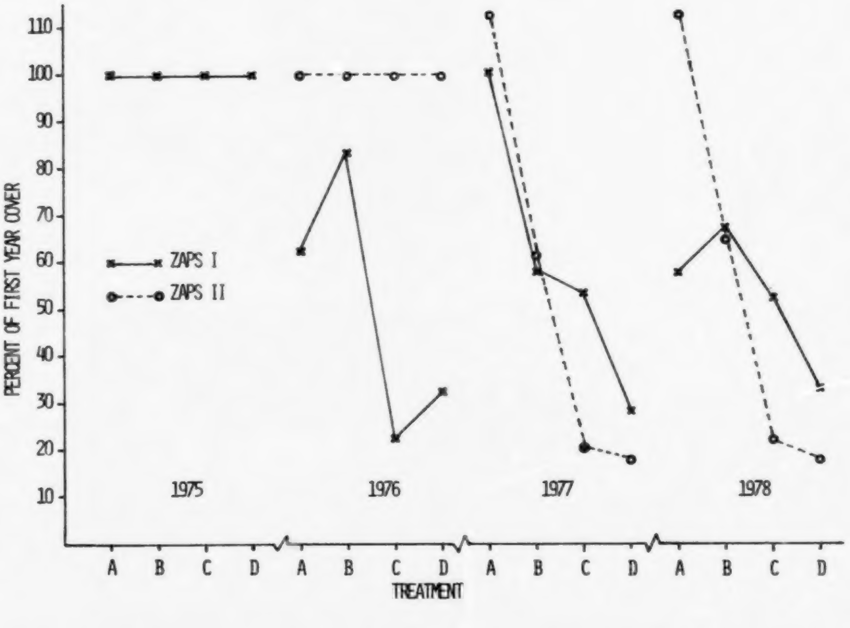

Figure 17.14. Lichen cover expressed as a percentage of first year cover, ZAPS I and II, 1975-1978.

Such changes now have been observed in several plant groups, as discussed above. If this trend continues, it will support the use of lichens as important bio-monitoring organisms.

Diversity

Diversity Index (H') values for 1978 are given in Figure 17.15. On ZAPS I at the first two observations all fumigated plots were significantly less diverse than the A plot. The low diversity on the B plot traces the pattern discussed earlier with respect to interplot variation. There is no obvious trend across the seasons, although the second observation, which coincides with the time of maximum plant growth, is a little higher. No consistent SO_2 effects are apparent.

The ZAPS II values are consistently lower than those of ZAPS I, and are more variable among treatments and dates. If any SO_2 effects are present they are masked by the inherent intrasite variation.

The evenness and richness values which contribute to the diversity of ZAPS I discussed above are shown in Figure 17.16. Even though diversity is more or less level in several instances, evenness and species richness can be seen to be changing in mutually compensatory ways. Evenness shows no clear seasonal or treatment trends. Richness tends to peak in mid-June, as is expected because of the active plant growth at that season. There are no consistent treatment effects, although mid-June and July may exhibit a down-trend with fumigation. All fumigated plots are significantly lower than the A plot at these times.

On ZAPS II evenness is much more variable than richness (Figure 17.17). The high evenness on C plot contributes most of the high diversity observed. Evenness is significantly higher than any other level in two of the four cases on C. Richness on D plot is significantly lower than all other levels at every observation. The B & C levels do not always differ significantly from the A plot.

Diversity on ZAPS I in 1978 compared to 1975 showed down-trends with fumigation in June and July, but not in September. September is a poor time to interpret diversity samples because many species present earlier have disappeared from the composition, leaving mostly the perennial dominants. Several of these earlier growing plants could be SO_2-sensitive. Also, late season composition varies with years depending upon the late season precipitation patterns. Thus, variation in fall vegetation is more a function of years than anything else.

Frequency

In a further attempt to characterize the nature of diversity changes among treatment plots, a frequency analysis was made (Figure 17.18). The theoretical basis of this analysis is derived from the work of Raunkaier (1934), who demonstrated a "J-shaped curve" of frequency distribution classes to be characteristic of many natural communities. Within each plot on ZAPS I,

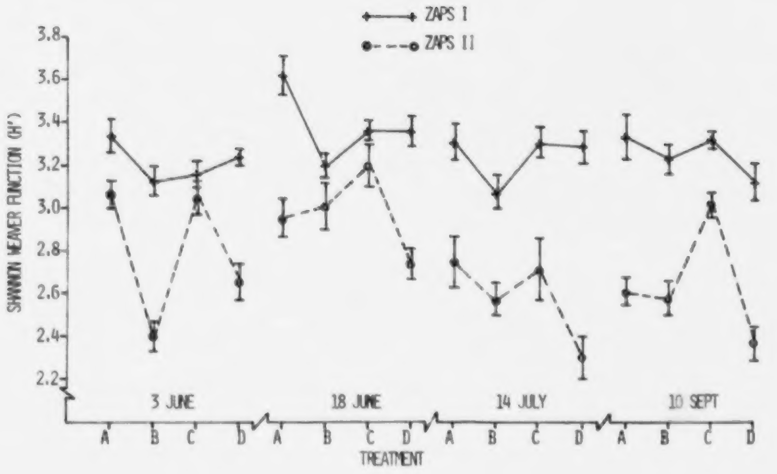

Figure 17.15. Diversity (means and standard errors) for ZAPS I and II for 4 sample dates, 1978.

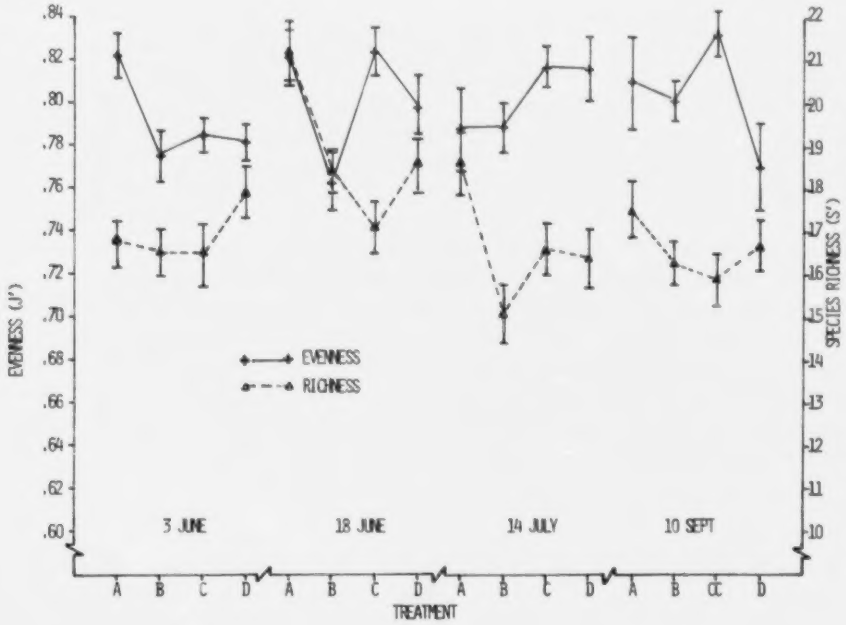

Figure 17.16. Evenness and species richness (means and standard errors) for ZAP I for 4 sample dates, 1978.

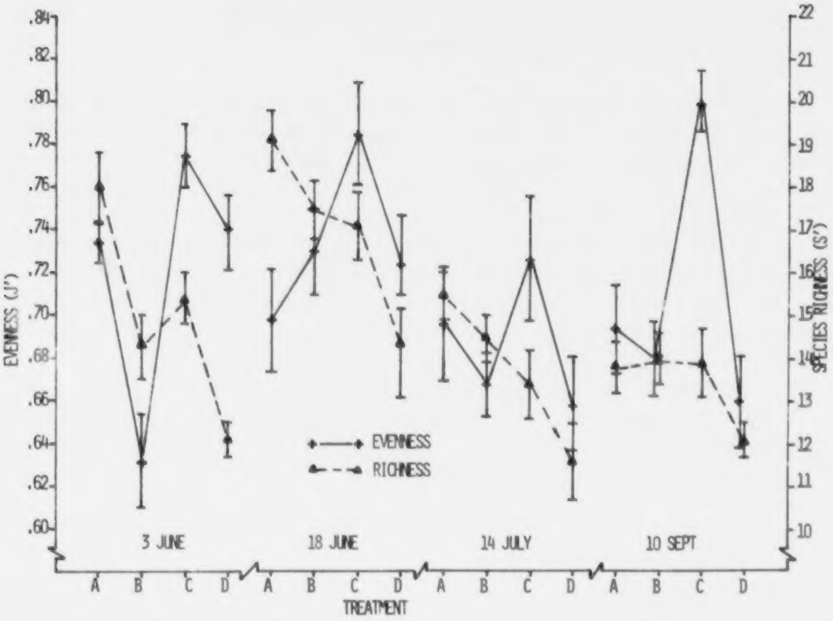

Figure 17.17. Evenness and species richness (means and standard errors) for ZAPS II for 4 sample dates, 1978.

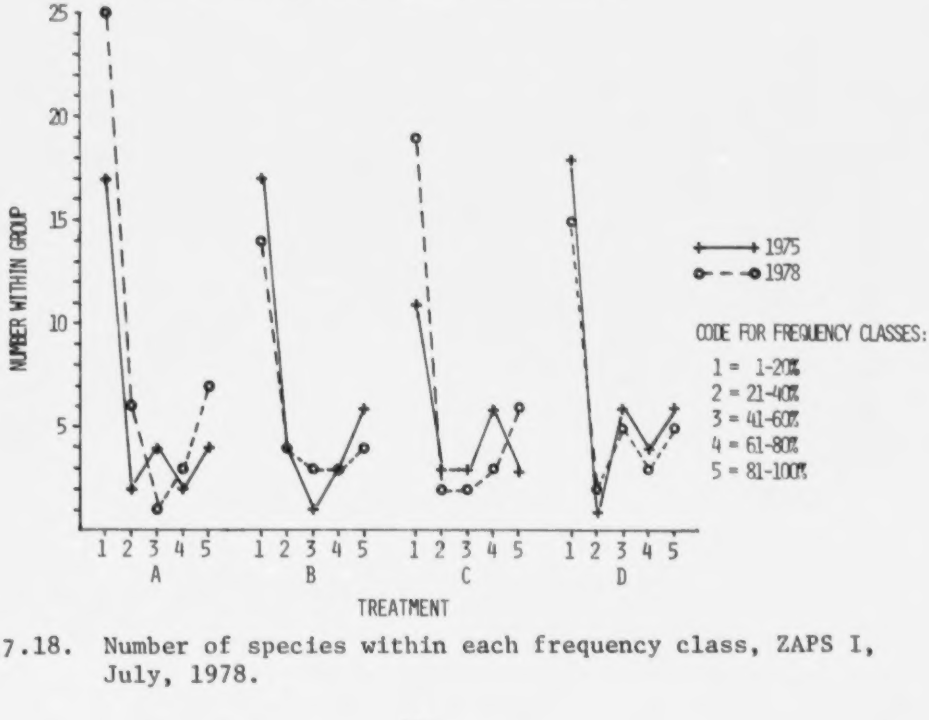

Figure 17.18. Number of species within each frequency class, ZAPS I, July, 1978.

the July data were used to construct frequency curves by plotting numbers of species as functions of percentage of the 2 x 5 dm canopy cover plots in which they appeared. Frequency percentages were divided into five equal classes, giving the curves presented.

If diversity changes are affected through either changes in species numbers or evenness, the shape of the frequency distribution should be altered. If dominant species are lost and not replaced, class 5 (81-100% frequency) should lose numbers. If rare or contagiously distributed species are removed from the composition, class 1 (1-20% frequency) will be depressed. Finally, if either or both of these circumstances should occur, the intermediate frequency classes may gain numbers. The net result would be a flattening of the whole curve, produced by the diminuation of end points and augmentation of the center.

The analysis is inconclusive, although some of the predicted curve changes may be happening. In particular, the B plot appears to be responding as suggested above. In 1979, both ZAPS I and II data from every sampling date will be examined in this way.

Western Wheatgrass Density

Western wheatgrass densities for 1975 and 1978 on ZAPS I A plot show little difference. With fumigation, though, almost all levels are significantly lower in 1978 (Figure 17.19). This should be expected after the similar pattern reported for cover. The ecological and economic significance of

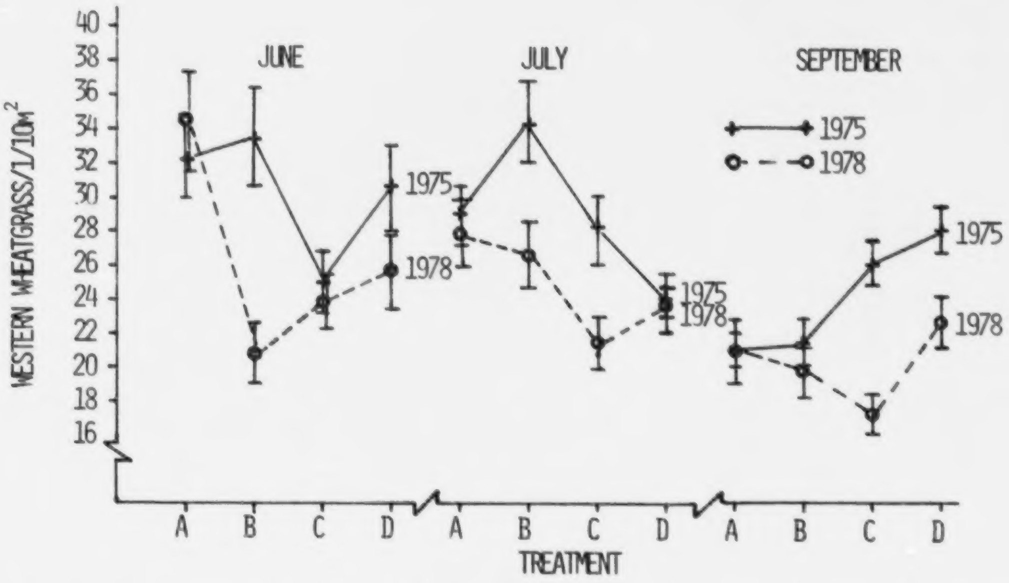

Figure 17.19. Western wheatgrass density (mean and standard errors) for ZAPS I, 1975 and 1978.

this change is substantial, since western wheatgrass is the dominant native forage species in the area, and is similarly important through most of the Northern Great Plains.

Photographic Monitoring

The ground photo plots were continued in 1978. They were photographed twice with color film, and charted in detail. The fertilization plots established by the Colorado State University team were photographed in stereoscopic color infrared once and color twice.

The aerial photography collected and analyzed over the ZAPS sites is discussed in Section 22.

CONCLUSIONS

Significant plant community changes have occurred in response to sulfur dioxide fumigation. Total living vegetation canopy cover on control plots generally increased, especially during the first three years. This probably is due to grazing deferment. Concurrently, fumigated plots, particularly the higher levels, decreased in cover. When 1978 data were compared with first year values, the control increased and the higher SO_2 rates decreased in cover. Graminoid cover was depressed at all fumigation rates. Much of this change was made by western wheatgrass, one of the most important forage species for livestock and wildlife in the region. Forbs and shrubs were too variable to show clear trends, but lichens were reduced by SO_2. Litter accumulated linearly for the first three years, apparently due to deferment and perhaps inhibited decomposition under fumigation. Litter cover leveled off the fourth year.

Neither diversity nor evenness were consistently sensitive indicators of fumigation stress, although some observations showed depressed values, at least at the higher SO_2 rates. Species richness was almost always lower on SO_2 plots.

Densities of western wheatgrass on ZAPS I in 1978 were significantly lower than 1975 levels on fumigated plots. This has considerable economic and ecological significance because of the importance of this plant as a forage species.

REFERENCES

Daubenmire, R. F. 1959. A Canopy-Coverage Method of Vegetational Analysis. Northw. Sci., 33(1):43-64.

Eversman, S. 1978. Effects of Low-Level SO_2 Stress on Two Lichen Species. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Third Interim Report, Colstrip, Montana. E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 385-398.

Eversman, S. 1979. Effects of Low-Level SO_2 on Two Native Lichen Species. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 642-672.

Pielou, E. C. 1969. An Introduction to Mathematical Ecology. J. Wiley & Sons, N. Y. 286 pp.

Raunkaier, C. 1934. The Life Forms of Plants and Statistical Plant Geography, Being the Collected Papers of C. Raunkiaer. Clarendon Press, Oxford.

Shannon, C. and W. Weaver. 1949. Mathematical Theory of Communication. Univ. Illinois Press, Urbana. 117 pp.

Taylor, J. E. and W. C. Leininger. 1979. Plant Community Changes Due to Low Level SO_2 Exposures. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana. E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 610-641.

SECTION 18

BEHAVIORAL RESPONSES OF SAPROPHAGOUS AND NECROPHAGOUS BEETLES EXPOSED TO SULFUR DIOXIDE

J. J. Bromenshenk

ABSTRACT

In 1976 and 1977, investigations at the Zonal Air Pollution System field study sites repeatedly demonstrated reduced numbers of saprophagous beetles captured in pitfall traps on plots treated with low level sulfur dioxide fumigation. This was particularly true for the tumble bug *Canthon*, mostly *laevis* Drury (Scarabaeidae). Pitfall trappings were again conducted in 1978 and 1979. In addition, experiments were initiated to determine whether the decreased abundance of beetles on the sulfur dioxide-treated plots was due to mortality, behavioral responses, or a combination of factors. Furthermore, quantification of the kinds and amounts of organic matter shredded and consumed by *Canthon laevis* was undertaken to estimate the effect of reduced numbers of these beetles on rates of decomposition, nutrient cycling, and regulation of dung and carrion-inhabiting pest insects. Preliminary evidence indicates that the observed low numbers of beetles on the sulfur-treated plots are due primarily to a behavioral reaction rather than to mortality. These scavanger beetles appear to be important removers of carrion and contribute significantly to the rapid removal of dung from grasslands of the Northern Great Plains.

INTRODUCTION

Data obtained in 1976 and 1977 at the Zonal Air Pollution System (ZAPS) study plots demonstrated that chronic low levels of sulfur dioxide (SO_2) significantly affected distributions of several different species and families of grassland beetles (Bromenshenk, 1979, 1978; Leetham *et al.*, 1979). Necrophagous, coprophagous, and predatory beetles reacted to sulfur dioxide, and tumble bugs or dung beetles of the *Canthon* group seemed to be particularly responsive. Repeatedly, more *Canthon*, mostly *laevis*, beetles were captured on the control plots than on any of the sulfur dioxide-fumigated plots. This depressed abundance may have been due to: (1) disorientation, avoidance, or otherwise altered direction or rate of locomotor activity; (2) natality or mortality of resident or transient populations, or (3) a combination of life table and behavioral factors. In 1978 and 1979, the primary objective of my research was to discover why fewer captures were made on the sulfur dioxide-treated plots.

Canthon laevis is considered to be a dung feeder, but observations at ZAPS indicated that these beetles also are voracious carrion feeders. Furthermore, the effective density of these insects in prairie grasslands is considerable. Typically, during ten-day trapping intervals from June to early August, hundreds to thousands of these beetles were captured on each of of the eight 0.62 ha plots.

Dung and carrion beetles are common in the grasslands of the Northern Great Plains, yet there is little information on their ecological roles in prairies. The major role of saprophagous invertebrates is assumed to be their contribution to the decomposition of organic material which in turn effects nutrient cycling. These organisms appear to function as regulators or catalysts of rates of decomposition by consuming, shredding, and burying organic matter, by altering chemical processes associated with decomposition, and by promoting the activities of microbial decomposers. However, few investigators have attempted to quantify the influence of these insects on decomposition rates.

Coprophagous and necrophagous beetles also compete for food and space with insects harmful to livestock and game animals, such as flesh flies and horn flies, and with potential transmitters of human disease such as muscid flies. In addition, dung and carrion-inhabiting beetles often carry mites and other insects that attack fly eggs. Other functions served by saprophagous beetles include less wastage of pasturage because of dung or carrion fouling and improving soil structure. They are also a food source for other organisms. Birds and amphibians may be their major predators. Australian investigators report that frogs, toads, magpies, starlings, plovers, crows, and other insectivorous birds feed on dung beetles (MacQueen, 1975; Rowley and Vestjens, 1973; Vestjens and Carrick, 1974; Green, 1966).

Verification of these functions, identification of other functions (if any), and quantification of arthropod-mediated rates of decomposition became major objectives of my 1978 and 1979 ZAPS research. Although these insects exhibit a response to sulfur dioxide, the significance of this response to ecosystems over the short and the long term remains to be determined. Also, beetles identified as being sensitive to sulfur dioxide represent only a few members of the invertebrate fauna associated with the biocenology of dung and carrion. Many other members of these communities have not been examined with regard to the effects of sulfur dioxide.

Feces and Carrion as Ecological Units

Feces and carrion each support a large and varied faunal community. Hayes (1927, 1929) commented, "One of the most interesting groups of prairie insects is the savenger group which originally derived its sustenance from droppings of the buffalo, but now thrives on the excrement of domestic cattle and horses which have replaced the buffalo on the prairies." Allee *et al.*,

(1949) remarked:

> "A neglected microsere is that of carrion. Decomposing bodies of fishes washed ashore, and the remains of dead reptiles, birds, and mammals, are especially well suited for research in this connection. Associated with changes in the chemistry of the flesh are numerous problems involving bacterial activities, carrion biocoenses, and the micoseral succession of the carrion fauna."

Hayes could not have known of the rapid spread and colonization success in dung of Hydrophilids of the genus *Sphaeridium* which are now very common in dung throughout the United States and Canada. These beetles apparently were introduced to the east coast of North America from Europe during the late 1800's or early 1900's (Brown, 1940). The concerns of Allee *et al.* have been addressed by Payne (1965) and others who have examined the carrion microcommunity.

Hammer (1941) cited Portchinsky (1885), a Russian scientist, as the first to consider cow droppings as ecological units, characterized by sharp competition among the insects inhabiting the dung and by predation of many insect species upon others. Hammer (1941) carried out extensive studies in Denmark concerning the biology and ecology of insects associated with cattle excrement, focusing his attention on Diptera (flies) but also examining Coleoptera (beetles), Hymenoptera (wasps), Collembola (springtails), and some non-insect arthropods.

Mohr (1943) seems to have been the first United States scientist to seriously consider cattle dung as an ecological unit. He conducted a study which still stands as one of the most detailed investigations of the entire insect complex of cattle droppings. Mohr documented the species of insects present and their succession, studied the physical changes that occurred in droppings from weathering and drying, examined competition and other relations among the flies, beetles, and parasitic wasps, and compared these phenomena at sites on sunny dry hillside pastures and on moist land in shady swamps in Illinois. He regarded bovine dung as a structural unit of prairie ecosystems and concluded that dung inhabitors apparently are "...not as common as general feeders on grass, weeds or shrubs, but are probably more common than many insects more often caught, but dependent on some specific grass, weed or shrub."

More recently, Sanders and Dobson (1966) determined the species, habits, and inter-relationships of insects in manure pads in a blue-grass pasture in Indiana. Coffey (1966) employed sites in Idaho and Washington to examine the potential for flies capable of carrying human disease to develop in cow, horse, sheep, chicken, swine, dog, mink, and human feces. Several studies in the United States, Canada, and Australia have examined supression of horn and flesh flies by populations of competitive or predatory insects inhabiting dung (Harris and Oliver, 1979; Blume, 1970; Blume *et al.*, 1970; MacQueen and Beirne, 1975; Thomas, 1967; Thomas and Morgan, 1972). Interest in dung beetles was engendered in Australia when cattle but not dung fauna were introduced. Problems developed such as excessive build-up of dung, epidemic populations of flies and parasitic worms, and excessive pasture wastage because of fecal fouling. In an attempt to alleviate these problems, dung beetles were introduced to Australia and intensively studied (Gillard, 1967; MacQueen, 1975; McKinney and Morley, 1975; Durie, 1975).

Currently, researchers interested in saprophagous invertebrates have reached the point where attempts are being made to quantitatively model the role of coprophagous fauna in the decomposition of organic matter. McKinney and Morley (1975) modelled dung and pasture interactions to assess the effect of dung beetles on the amount of area of unfouled pasture accessible to grazing, on soil moisture, and on nutrient storage, especially of phosphorus and nitrogen. Studies by Olechowicz (1974, 1976, 1977) and Andrzejewska (1974) on decomposition rates of dung, the contribution of invertebrate fauna to this process, the amounts of dung deposited, green biomass production, and sheep consumption have been used to construct a diagram of energy flow through the main food chain of sheep grazing a mountain pasture. The conclusion of these studies was that coprophagous animals play an important part in the rapid decomposition of sheep feces and its "fast recycling." After 30-day exposure periods, an average of 35.3% of the energy in dung dissipated. Invertebrates accounted for 61.4% of the dung loss, while the microorganisms processed 38.6%. In other words, dung accessible to invertebrates had a decomposition rate twice as high as that of dung subject only to the action of microorganisms. Whether the same was true of cattle droppings in grasslands of the Northern Great Plains became the pragmatic basis for my 1978-79 ZAPS studies.

MATERIALS AND METHODS

Pitfall Traps

In 1978, I continued population studies using pitfall traps arranged in grid arrays across the treatment plots. Each consisted of a cone-shaped, disposable, plastic coffee cup set into 14 oz. plastic beverage cups and dug into the ground so that the lip of the coffee cup was flush with the soil surface. The traps are based on the design of Morrill, (1975) and modifications by Olson *et al.*, (1976) and Bromenshenk, (1978). An inch of water in the bottom of each trap drowned captured beetles, prevented predaceous species from devouring each other and other captured insects, and kept the bait moist. All traps were baited with five grams of meat. Baiting increased trapping success for Scarabaeids and Silphids and resolved an over-trapping problem caused by traps containing beetles acting as attractants as compared to unbaited traps without beetles. Each grid formed a 7 x 7 square, the perimeter traps located outside the pipeline system of each treatment plot (Figure 18.1). Thus, at each plot there were 49 traps, 25 within the gas delivery lines, 24 outside of the delivery lines or 392 traps in all. The main purposes of using these traps in 1978 were verification of the 1976 and 1977 findings and capture-recapture studies. Tending the traps and conducting counts of captures was very time consuming. Therefore, in 1978 traps were used only in mid-summer (July) at a time when the beetle populations, based on our previous findings, would be at peak levels of abundance.

The use of pitfall traps and the baiting of pitfall traps is controversial among investigators. The main concern is that differences of species susceptibility to capture, attraction of animals from a distance, and trap shielding by dense vegetation seriously limit the ability to obtain accurate estimates of absolute population density. However, pitfalls are generally thought to be useful for studies of relative abundance, movements, spatial patterns of distribution and diel activity patterns, if the plots occur in similar vegetation. This sample method is simple, easy to use, relatively inexpensive, and may not have any more problems than other methods such as direct quadrant counts, total population per area, or mark-capture (Kulman, 1974). More detailed discussions of the advantages, disadvantages, and limitations of pitfall trapping can be found in Southwood (1975), Kulman (1974), Thomas and Sleeper (1977), and Bromenshenk (1979).

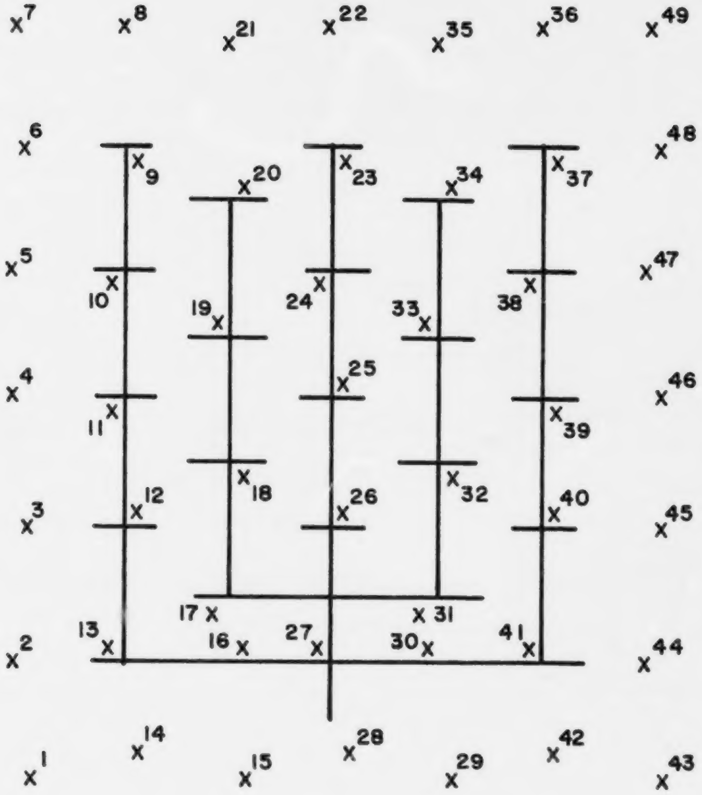
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Figure 18.1. Trap locations for each treatment plot.

Shubeck Traps

In 1979, a new trap design was utilized. Separating beetles from a slurry of decomposing meat in water was putrid, and mark-recapture tests conducted in 1978 indicated that markings with paint peeled off of the beetles in the traps. Shubeck's (1968) trap was a ground-surface trap containing the bait in a cup set into a one-gallon can which was capped by a wide mesh hardware cloth and housed in a wooden box with a canopy roof to keep out rain. Thus, the trap was relatively clean, dry, and efficient to use. Shubeck (1976) reported good success in collecting beetles representing several families: Silphidae, Scarabaeidae, Staphylinidae, Histeridae, Leiodidae, and Nitidulidae. But for my purposes, this trap was considerably more complex and expensive than the pitfalls (@ $5.00/trap versus 4.5¢/trap), especially when large numbers of traps were to be used. Rain was a major problem to Shubeck but of little importance at the ZAPS, since during mid-summer generally the only precipitation was from infrequent thunder-showers. Therefore, I utilized a modification of Shubeck's original design (Figure 18.2). A plastic food container (pail) was fitted with a wire mesh screen nailed to a wood frame. The frame was attached by an L-shaped, ¼" bolt and wing nut to a steel rod, in order to secure the trap from being knocked over or removed by coyotes or wind. A beverage cup set into the pail held the bait.

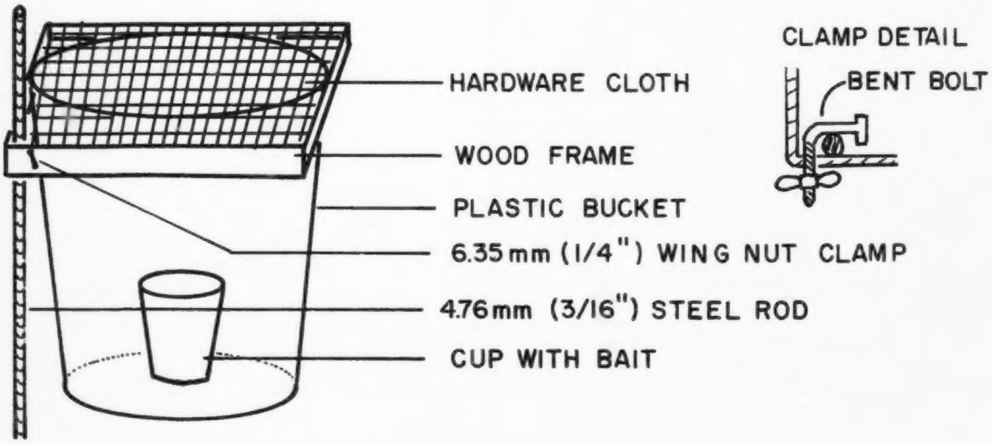

Figure 18.2. Modified Shubeck trap.

One of these traps was placed beside each of the poles used by EPA to hold sulfation plates for an extensive study of the general relative concentrations of sulfur dioxide at different horizontal and vertical locations on the plots

(Preston and Gullett, 1979). Each pole held sulfation plates in clips at four heights. One pole was located in the center of each quadrant of each treatment plot and each internal plot. A fifth pole was placed in the geometric center of each internal plot. The fifth trap on each treatment plot was placed 2 m from the geometric center of the plot to avoid placement directly under a gas delivery line (Figure 18.3). This differed from the location of the fifth pole which was placed next to the real-time sulfur analysis sample position c. Two posts and traps were located in the buffer zones at each end of the series of plots.

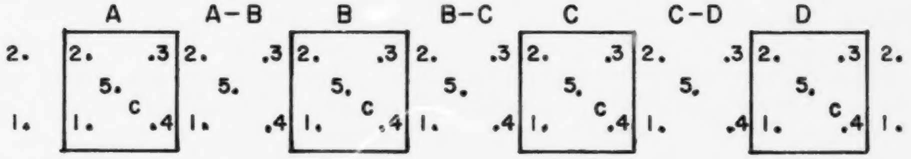

Figure 18.3 Location of Shubeck traps for beetle capture on the extensive grid.

Mark-Recapture Tests

During 1978 and 1979, beetles of *Canthon laevis* were marked and released on the ZAPS plots. The main objective of the 1978 test was to monitor movement as regards direction and distance. The intent for 1979 was to conduct life-table tests using mark-recapture techniques,but until late in the summer population levels were so low that both total numbers and recapture success were too small for meaningful interpretation of the results. Beetles were marked in three ways: (1) dots of acrylic paint on the thorax, (2) tarsal clipping, and (3) branding of the thorax with a hot needle.

Paint markings were rapidly lost probably because the beetles are strong diggers, descending below the soil surface to avoid temperature and other climate extremes and to bury their dung balls. Thus, the paint is literally scraped off. In pitfall traps, water dissolved the paint. Tarsal clipping was permanent, but extremely difficult to see, especially in traps containing hundreds of individuals. Branding also was permanent but has a greater chance of injury. Paint appeared to suffice for short-term movement evaluations, if dry traps such as the Shubeck design were utilized.

Mouse Carrion Study

In 1978, a study of the decomposition of mice was conducted at ZAPS. Mice were placed in two types of cages on each of the ZAPS treatment plots and at an additional control plot near the Rocky Boy Reservoir about two miles from either ZAPS site. The first type cage consisted of a bottomless box of galvanized hardware cloth (1.2 cm spacing of the mesh wire) with long tabs at the sides which were dug into the ground to secure the cage. Each cage was rectangular and just large enough to contain a mouse. Thus, each mouse carcass rested on the surface of the soil with hardware cloth covering it. Mice in these cages were more or less protected from mammals and birds but still accessible to the beetles which could easily pass through the mesh. The second type of cage consisted of a metal window screening (2 mm spacing of the mesh) formed into a rectangular box just large enough to hold a mouse. These cages totally enclosed the carcasses and all seams were folded over and stapled for added security. The mesh size was small enough to exclude macroarthropods. After an early season test at ZAPS, it became apparent that many small arthropods still had access to these carcasses. In later tests, each carcass was wrapped in four layers of cheesecloth before being put into the screen cage.

Two tests of carrion decomposition were carried out in 1978. Each utilized 90 mouse carcasses. In the first test, the mice were obtained in June from the Western Energy's mine reclamation areas near Colstrip. Two nights of snap-trap captures were carried out. Captured mice were removed from the traps just after sunrise, and the bodies frozen until used. Ninety deer mice (*Peromyscus maniculatus*) comprised of 45 females and 45 males were selected for the ZAPS test. Each frozen mouse was weighed and the fresh weights recorded. Specimens of each sex were arranged in order by weight, then segregated and ranked into five weight categories. Using a table of random numbers, nine male and nine female mice were drawn from each weight grouping and assigned to a treatment plot. This provided five females and five males for each plot. Sets of ten mice were separated into male/female pairs, each individual of a pair of similar weight. Thus, for each treatment there was one pair of small mice, one pair of somewhat larger size, and so forth. The series ended with a pair of the largest mice. One individual of each pair was placed into a screen cage, the other into a hardware cloth cage. The choice of which sex to place in which cage was again randomized using the table of random numbers.

As mentioned before, on each treatment plot, there are five posts for holding sulfation plates arranged in an x-shaped pattern. One pair of caged mice was placed as near as possible to each post. The selection of which of the five mouse pairs to put by a post was made from the random numbers table. Thus, at each post was placed a male/female pair of mice of similar weight, one individual in a screen cage and the other in a hardware cloth cage. Thus, weight and sex factors were thoroughly randomized for each treatment. The cages were examined frequently for several days following placement on the plots and observations made concerning insect activity and decomposition. After 60 days the carcasses or whatever remained of the mice were retrieved from the plots, the debris cleaned away, the remains oven dried for 97 hours at 50°C, and weights recorded. The soil under each hardware cloth cage was removed to a depth of 30 cm and sifted through a fine mesh screen in order to remove any buried material. A second set of 90 mice was utilized in a test begun in September, 1978. It differed from the June test in four ways: (1) white laboratory mice instead of deer mice were utilized, (2) mice were not segregated on the basis of sex, (3) carcasses placed in the screen cages were wrapped in cheesecloth, and (4) carcasses were exposed for 12 months.

White mice were used because they were much more readily obtained than deer mice. The mice were not separated by sex because 45 of each sex of mice were not available and because no sex-related effects had been observed during the June test.

Bovine Feces Decomposition

In 1979, a study of the decomposition of cattle feces was begun at ZAPS. Based on the experiences with the 1978-79 carrion tests, it became apparent that it is difficult if not almost impossible to effectively exclude scavenger arthropods. Powerful shredding and biting mouthparts, digestive enzymes, and efficient strategies of obtaining their food when difficult to reach lead eventually to at least the smaller arthropod species gaining access. Watching *Canthon laevis* on cow pats made it apparent that their feeding and ball rolling activites could be easily recognized by a visual examination of the dropping. Dung loss attributable to these beetles can be estimated by the area of the pat disturbed or removed. Since the primary objective was to determine if the observed reduction in the numbers of *Canthon laevis* on the sulfur dioxide treated plots would alter rates of decomposition and since virtually nothing was known about the responses of the total decomposer communities of dung to sulfur dioxide or whether a change in the numbers of *Canthon laevis* might be offset by concurrent changes in the population dynamics of their competitors, I elected to concentrate on the responses of the entire decomposer community to sulfur dioxide. To accomplish this, 16 standardized cow pats were placed on each plot at the ZAPS in early and late summer, 1979.

Fresh bovine droppings were obtained from a concrete pad of a holding pen of a dairy operation at Billings, Montana. The concrete was washed down before the cattle were admitted, the cattle held for two-hour periods and then released. The fresh dung was shoveled into 30-gallon garbage cans lined with plastic bags. The cans were then sealed and the manure transported by truck to the ZAPS. At ZAPS, each can was thoroughly stirred using a long-handled shovel. Then 14 oz. disposable, plastic beverage cups were filled level with the top with dung. A trowel was used for the dung transfer to the cups. Sets of 18 cups were filled. From these, two were randomly selected for immediate weighing (fresh weight) and subsequent oven-drying and weight determinations. From the remaining 16 cups, groups of four cups were drawn randomly and assigned to one of the four treatment plots at each ZAPS. While one individual filled and weighed cups, another carried the groups of four to the plots and set them on 4 x 4 grids, laying a line of four cups in an east-west direction on each plot. After one set of four had been placed on each plot, the procedure was repeated until 16 per plot had been delivered. The grids were marked off before filling the cups by placing a 24.2 cm paper plate at each grid intersection point. The contents of the cups were emptied onto the plates and spread with a trowel into a symmetrical pat approximately 3.5 x 20 cm (Figure 18.4).

Insect activity was observed immediately following placement of the dung on the ZAPS. In July, the number of insect-produced holes in the top and bottom of each pat, the area removed, and the field dry weights were recorded for the two early season trials. The "dry weights" were obtained in the field in late July on hot, sunny days with the temperature over 24°C and the relative humidity less than 30%. All dung pats were replaced in the original positions after performing the counts, area determinations, and weighings. A third set of dung pats was placed on the ZAPS in September. A subset of these pats was enclosed in mesh cages to exclude the larger invertebrates. All pats will be removed following shutdown of the gas delivery in October, 1979. These will be transported to the EVST Laboratory, oven-dried, and final measurements conducted. Two-way ANOVA (analysis of variance) of the field dry weights, emergence and entry holes, and area of dung removed will be carried out.

Figure 18.4. Standard cow pat on paper plate.

Besides investigating sulfur dioxide effects on rates of dung decomposition, it was hypothesized that the dung pats might provide a means of assessing the activities of insect population by the number of feeding and emergence holes in the pats. It was hoped that this might provide a useful tool analagous to moss bags.

Tube Test of Beetle Movements and Orientation

Fewer numbers of beetles on sulfur treated plots may be due to a behavioral response such as disorientation, altered rates of locomotor activity, impairment of ability to find dung or carrion, or directed movements such as caused by an irritant or a repellant. In order to examine this in a field setting, 12 cylindrical wire mesh tubes (0.3 cm spacing of wires) 9.5 x 4.57 cm were constructed. An entry was provided at the middle of each tube. The tubes were marked off into five equal-length sections.

The use of the tubes was as follows: Sets of tubes were placed on the A and D plots at either ZAPS I or ZAPS II. For each test, the tubes on A and D were similarly placed with respect to the gas delivery lines, sun, slope, and wind. An observer was situated near the tubes on each of the two treatments. At precisely the same time, each observer would release 20 beetles into each tube and then record at five minute intervals for 30 minutes the position of each beetle. At the end of 30 minutes the beetles were removed from the tubes. Tests in which sulfur dioxide was excluded as a factor were conducted off the plots. By various arrangements and orientations of the tubes, factors such as slope, light, food odors, wind, and sulfur dioxide could be examined individually and in combinations. Obviously, this procedure was best suited to investigating tactic responses rather than kinetic. Kinetic experiments were also carried out at ZAPS in 1979. They will be described in the Sixth Interim Report, and only preliminary results of the tube tests will be given here.

RESULTS

The results of the 1978 investigations are presented here, as are some of the 1979 results which either are a continuation of the 1978 work or preliminary information from the 1979 season which facilitates discussion of the hypotheses and objectives.

Summaries of the captures of all species of beetles in pitfalls at ZAPS I and II for June-July, 1978 appear in Appendices 18.1 through 18.9. Captures of *Canthon laevis* for the ten-day period totalled 28,076 beetles, 2.7 times greater than for any prior sample period. Total capture for ZAPS I exceeded peak season captures for 1977 by 4.76 times, while total captures at ZAPS II were 1.25 times that of 1977. In 1976 and 1977, captures at ZAPS I were always lower than at ZAPS II. As in previous years, capture success on all of the sulfur dioxide treated plots as compared to the "controls" (A plots) was significantly lower ($p \leq 0.005$) (Figure 18.5) by Chi-Square Goodness of Fit Analysis (Table 18.1). This was true for total captures, perimeter captures, and interior captures. On ZAPS I, there was no significant difference for interior captures on the B or C plots as compared to D.

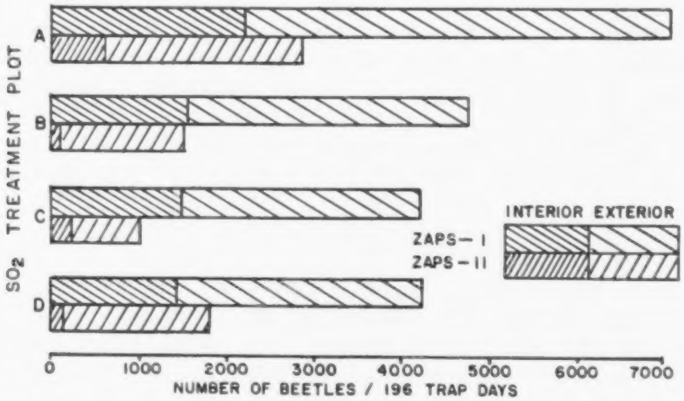

Figure 18.5. Pitfall captures of *Canthon laevis* at ZAPS I and II, June-July, 1978.

TABLE 18.1. CHI-SQUARE GOODNESS OF FIT FOR PITFALL CAPTURES OF *CANTHON* SP. AT ZAPS I AND II, 1978

	Total		Perimeter			Interior		
Plots	$p \leq .05$	$p \leq 0.005$	N.S.	$p \leq 0.05$	$p \leq 0.005$	N.S.	$p \leq 0.05$	$p \leq 0.005$
					ZAPS I			
ABCD		958.4			703.0			299.1
AB		415.8			222.5			207.8
AC		677.1			549.5			148.7
BC		16.4			75.4		6.6	
BD		14.1			27.6	1.3		
CD	3.9				11.9	2.03		
AD		580.3			403.5			176.9
BCD		34.3			77.4		6.6	
					ZAPS II			
ABCD		1045.4			741.3			629.5
AB		427.6			190.8			398.4
AC		904.1			729.4			176.6
BC		101.0			191.4			64.0
BD		25.8			17.6			15.0
CD		226.0			319.6			18.3
AD		248.0			93.6			289.4
BCD		225.6			324.8			39.8

Although there were significant differences in the numbers of other species of beetles for plots at ZAPS I and II as indicated by Chi-Square Goodness of Fit Analysis, none of the differences correlated with any discernible sulfur dioxide treatment effect. However, for the most part, captures of beetles other than *Canthon* species were relatively low.

Preliminary tests of the Shubeck traps in June, 1979 produced numerous captures of Silphids of the genera Nicrophorus and Silpha but almost no other insects. Setting the traps into the ground flush with the soil surface immediately resulted in captures of *Canthon* species and of other species of beetles which frequently had been caught in the 14 oz. pitfalls. Results of the 1979 trapping will be presented in the Sixth Interim Report, 1980.

Mark-Recapture

Mark-recapture tests were conducted in 1978 during the June-July trapping interval. Additional tests were conducted in 1979, the results of which will be presented in the Sixth Interim Report. In 1978, 528 *Canthon laevis* were marked with acrylic paint and released on ZAPS I and II--400 at ZAPS I and 128 at ZAPS II. Beetles were obtained from the pitfalls on the second day of the ten-day trap interval, marked, released on the third day, and total recaptures after seven days (tenth day of the trapping interval) determined. The released beetles were color-coded and tarsal-clipped by plot. Forty-eight hours after capture, two sets of beetles were released on each plot at two release points. Each set was designated by a distinctive pattern of the markings. Fifty beetles were released at each point on ZAPS I and 16 at each point on ZAPS II, or a total of 100 per plot at ZAPS I and 32 per plot at ZAPS II.

Recaptures, as indicated by colored markings, were very low--45 beetles or 11.3% for ZAPS I, and 17 beetles or 13.3% at ZAPS II. However, most of the markings on the beetles in the traps dissolved, so that the recapture numbers indicated the number of traps with paint chips in them. It is possible to obtain a better estimate from the clipped tarsi, but detecting these on a small number of beetles out of more than 28,000 beetles is extremely difficult, although it is currently being attempted.

Marking tests performed on 400 *Canthon* beetles kept in two cages at the University of Montana's Botany Gardens, Missoula, Montana, revealed that the digging activities of these beetles effectively removed any type of paint or tags within hours to a few days. Clipping of any part of the body other than the tarsi either resulted in hindrance of beetle activities or injured the beetles. For example, the thorax is so hard that attempting to clip the margin often results in shattering the carapace. Clipping the elytra interfered with flight. During the winter of 1978, branding the prothorax with a hot needle was found to result in a permanent mark, easier to discern than clipped tarsi, and both survival and activity did not seem to be appreciably altered. Trapping in June and July of 1979 resulted in relatively low numbers of *Canthon*. In August, trapping success increased and some mark-recapture tests were performed, the results of which will appear in the next report.

Although the mark-recapture data for 1978 is of little or no value for interpretation of population dynamics and life-table factors due to loss of markings and due to apparent low recapture numbers, useful information was obtained concerning movements. Release and recapture positions are indicated in Figure 18.6. As expected, the greatest number of recaptures as indicated by the presence of paint in the traps were at the traps nearest the release points. However, beetles released on the A plot of ZAPS I were captured as far away as D plot and vice versa. Similar movements were observed on ZAPS II. Movements of distances greater than 430 meters occurred. Movements of 250 to 300 meters were common. Upon release, some beetles ran away on the ground, but many took to the air and flew off. Frequent observations of beetles in flight at ZAPS in 1978 and 1979, both upon release and when approaching bait or dung, have shown *Canthon* beetles to be strong and swift fliers, capable of traversing a plot in a few seconds, able to hover in place, and able to land with almost pinpoint accuracy. *Canthon* beetles have been observed flying behind an investigator carrying bait. They have been seen to hover approximately 0.5 m above a pitfall trap, suddenly fold in their wings, and drop straight into the trap without touching the sides of the coffee cup. More often, they fly in a zigzag course towards dung or bait, land nearby, and scramble across the ground to their objective. When taking to flight, most crawl to the top of the vegetation canopy on leaves or stems before launching. Attempts to take off from the ground usually end by crashing into the vegetation. Occasionally, beetles took to flight from areas of bare ground or from dung pats. Flight activity appears to be affected by meteorological conditions. When strong winds occur, these beetles are seldom seen in flight but continue to move about on the ground. Cool temperatures and very hot temperatures seem to depress flight activity. Most flight activity seems to occur at air temperatures from 16°-29°C, based on field observations. Caged beetles generally burrowed under vegetation or soil when temperatures exceeded 29°C or dropped below 10°C. These temperature limits to kinesis are being investigated in detail, the figures obtained to date are approximations, and the thresholds have not been determined accurately. It is apparent that my first presumption, based on trap data, that these beetles are active at night may not be correct. They are very active during the daytime and demonstrate actions suggesting temperature and possibly photic responses.

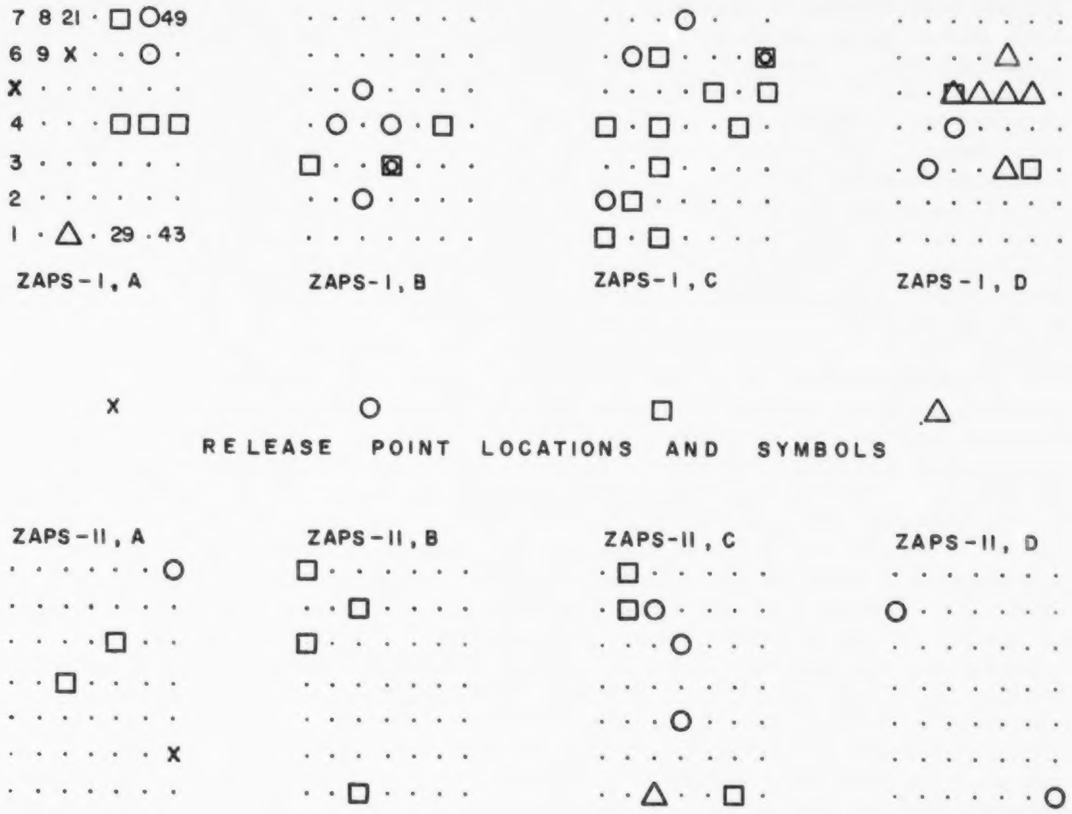
7 8 21 49
6 9 X
X
4
3
2
1 29 43
ZAPS-I, A
ZAPS-I, B
ZAPS-I, C
ZAPS-I, D
X
RELEASE POINT LOCATIONS AND SYMBOLS
ZAPS-II, A
ZAPS-II, B
ZAPS-II, C
ZAPS-II, D
X

Figure 18.6. Recapture points of painted beetles.

Although most beetles lost their paint markings rapidly, a few did not. In July of 1979, the body of a painted beetle was found in one of the dung pats used in the dung decomposition tests. This beetle had been marked and released a full year prior to the setting out of the dung. Although it is possible that something deposited the body of the beetle on the dung such as a bird, small animal, or wind, the position of the body suggests that the beetle was alive and active and attempted to burrow into the pat before expiring. If so, then the beetle had lived for more than a year at ZAPS.

Mouse Carrion Study

The first carrion study was conducted from June 26, 1978, to September 16, 1978; the second from September 16, 1978, to September 16, 1979. Thus, only the data from the first test was completed for this report. Fresh and dried weights are given in Table 18.2. The remainder will be in the Sixth Interim Report.

Carrion exposed to macroarthropods in the hardware cloth cages was rapidly found by carrion beetles (mostly *Nicrophorus* species), tumble bugs (mostly *Canthon laevis*), and ants (mainly harvester ants). The carrion beetles buried several carcasses within two to three hours of placement on the plots. The tumble bugs rolled away flesh balls, tearing and shredding the flesh, especially in the abdominal region. On the Off Plot Control, ants arrived at the carcasses almost immediately and attacked any other invaders such as carrion beetles and tumble bugs. Within 48 hours, tufts of hair, bones, and a few dried pieces of flesh were all that remained of 43 out of 45 specimens. The ants took several days to thoroughly strip a carcass. Of the 45 carcasses, 82.2% were buried by carrion beetles, 15.6% stripped by ants (6.7%) or tumble bugs (8.9%), and 2.2% stripped by other invertebrates. By the end of the trial, occasional bones and tufts of hair of the buried carcasses were found. In most cases, nothing was found even after sifting the soil through a fine mesh screen. The carcasses in the screen cages underwent a slow drying out process, and after two to three weeks, it became apparent that microarthropods such as mites and small ants had invaded the abdominal regions. By the end of the trial, these carcasses had mummified with bones, hair, and skin still more or less intact. Carrion beetles, tumble bugs, and ants removed the majority of organic material; other invertebrates such as skin and hair beetles, collembolans, flies, and mites consumed smaller amounts. Assuming 67% water content for the body of a mouse, 94.37% of the carrion (dry weight basis) was removed when large insects were present. Microorganisms decomposed 63.97% of the organic material. The larger insects removed major masses of tissue within a few hours, while microorganisms took several weeks before discernible changes in the carcasses occurred. In addition, at the end of the trial, bones, skin, and hair remained where microorganisms were the only scavengers and decomposers, but in over 80% of the cases even the bones were gone when large insects had access.

In terms of total biomass, six times as much organic material remained of the carcasses exposed only to microorganisms compared to those accessible to the large insects.

TABLE 18.2. FRESH AND DRY WEIGHTS* OF MOUSE CARCASSES BY PLOT, SEX, CAGE AND POLE POSITION

	Fresh Weight				Dry Weight			
	Hardware Cloth Cage		Screen Cage		Hardware Cloth Cage		Screen Cage	
Pole	(Sex)	(Weight)	(Sex)	(Weight)	(Sex)	(Weight)	(Sex)	(Weight)
Off Plot Control								
1	Male	22.2	Female	31.2	Male	0.60	Female	3.47
2	Male	17.4	Female	20.6	Male	0.13	Female	1.93
3	Female	22.6	Male	19.9	Female	0.88	Male	3.30
4	Female	23.0	Male	21.5	Female	0.00	Male	1.82
5	Female	13.4	Male	10.8	Female	1.80	Male	4.27
ZAPS I, Plot A								
1	Male	24.8	Female	26.8	Male	0.00	Female	2.80
2	Male	20.5	Female	23.1	Male	0.00	Female	2.73
3	Female	21.3	Male	16.9	Female	0.00	Male	3.91
4	Female	22.9	Male	18.3	Female	0.00	Male	1.57
5	Female	13.7	Male	14.3	Female	0.00	Male	2.20
ZAPS I, Plot B								
1	Female	25.4	Male	23.3	Female	0.00	Male	1.91
2	Male	21.5	Female	24.6	Male	0.00	Female	2.02
3	Female	22.6	Male	19.0	Female	0.00	Male	2.31
4	Female	17.0	Male	19.0	Female	0.00	Male	3.14
5	Male	15.8	Female	15.1	Male	0.00	Female	3.48
ZAPS I, Plot C								
1	Female	27.1	Male	23.4	Female	0.00	Male	1.40
2	Male	15.8	Female	17.5	Male	0.00	Female	2.17
3	Female	20.2	Male	16.2	Female	0.60	Male	2.06
4	Female	25.2	Male	21.8	Female	0.00	Male	3.27
5	Male	19.3	Female	23.0	Male	0.00	Female	1.84
ZAPS I, Plot D								
1	Male	25.7	Female	29.1	Male	0.50	Female	2.42
2	Male	21.2	Female	25.4	Male	0.00	Female	2.91
3	Male	19.8	Female	21.6	Male	0.00	Female	2.53
4	Female	21.0	Male	17.7	Female	0.00	Male	2.16
5	Female	15.5	Male	15.7	Female	0.80	Male	2.49
ZAPS II, Plot A								
1	Female	35.5	Male	22.1	Female	0.40	Male	2.33
2	Female	25.3	Male	21.4	Female	0.30	Male	1.97
3	Female	23.3	Male	18.3	Female	1.20	Male	1.54
4	Male	16.8	Female	19.2	Male	1.35	Female	1.55
5	Male	16.0	Female	15.8	Male	0.40	Female	1.66
ZAPS II, Plot B								
1	Female	29.0	Male	23.8	Female	2.70	Male	2.58
2	Female	24.0	Male	22.0	Female	0.30	Male	2.43
3	Male	18.7	Female	23.0	Male	0.00	Female	2.69
4	Female	20.9	Male	17.2	Female	0.70	Male	1.83
5	Male	15.7	Female	13.0	Male	0.00	Female	2.68
ZAPS II, Plot C								
1	Female	31.3	Male	23.3	Female	0.50	Male	2.84
2	Male	20.7	Female	25.0	Male	0.80	Female	2.39
3	Male	18.3	Female	22.3	Male	0.00	Female	1.33
4	Male	17.8	Female	21.2	Male	0.00	Female	1.79
5	Female	14.4	Male	13.2	Female	0.00	Male	1.77
ZAPS II, Plot D								
1	Female	21.9	Male	19.4	Female	0.40	Male	2.04
2	Male	15.8	Female	14.0	Male	0.00	Female	2.88
3	Female	18.7	Male	16.9	Female	0.75	Male	1.37
4	Female	24.0	Male	21.1	Female	1.30	Male	3.67
5	Male	26.0	Female	26.7	Male	0.60	Female	2.51
All Plots								
		$\Sigma = 949.05$ $\overline{X} = 21.09$ $S_x = 4.70$ $S_{\overline{x}} = 0.70$		$\Sigma = 914.70$ $\overline{X} = 20.33$ $S_x = 4.38$ $S_{\overline{x}} = 0.65$		$\Sigma = 17.61$ $\overline{X} = 0.37$ $S_x = 0.57$ $S_{\overline{x}} = 0.08$		$\Sigma = 108.76$ $\overline{X} = 2.42$ $S_x = 0.69$ $S_{\overline{x}} = 0.10$

* Grams

In September, a second trial was performed. Care was taken to exclude microarthropods more effectively by wrapping the mice in layers of cheesecloth. The tests were run for an entire year. As before, beetles and ants began working on the bodies within a matter of minutes or a few hours. On ZAPS I, a large animal, probably a coyote or fox, dug up and removed the bodies from 12 of the 20 hardware cloth cages. It appeared that the animal worked across the plots taking four mice at A, four at B, three at C, and one at D. Final results for the trial will be presented in the Sixth Interim Report.

Bovine Feces Decomposition

Fresh and oven-dried weights of 415 cm^3 of bovine feces are presented in Table 18.3. Both wet and dry weight means demonstrated a low degree of variability, coefficients of variation ranging from 1.3%-5.1%.

Soon after placement on the plots, the cow pats began to attract insects. *Canthon* beetles arrived as soon as 5 minutes after setting out a plate, although most arrived 20 minutes to a few hours later. *Canthon* continued to arrive for 2-3 days, if the interior of the pats remained moist and semi-liquid.

One of the first and most common visitors to the dung were *Sphaeridium scrabaeiodes* Linnaeus which arrived at the dung almost as soon as it was set out and continued to arrive for 3-4 hours, most appearing within the first hour. Mohr's description (1943) of hydrophillid activity is germaine: "Most of them arrive during the first half hour, run excitedly over the surface, plunge inside and come out again to repeat the performance many times."

Flies and early visitors were abundant. Horn flies (*Haematobia*), false bottle flies (*Cryptolucia*), sarcophagid flies (*Sarcophaga*), minute dung flies (*Leptocera*), and others were common on the fresh dung, and fly larva were numerous in the dung by the second or third day.

Other scarabaeid beetles, particularly *Aphodius* species arrived at the dung and immediately burrowed into the interior. These beetles were easiest to detect by breaking open one-day or older pats. Many mites were observed on the surface of the droppings, many arriving on the beetles.

The competition for the dung resource appears to be sharp. Time of day, temperature, wind, moisture content of the pat, and time of year were but a few factors which seemed to affect the succession of insects at the feces. During the summer of 1979, T. J. McNary, Colorado State University, performed tests on the kinds and numbers of insects in the microseral stages, and on the effect of time of day on insect succession. This information should be available for incorporation into the Sixth Interim Report.

During mid-July, some of the pats set out in early summer had the entire middle area removed so that only a ring of dung remained. Some were extensively hollowed out from underneath, while others had large portions missing out of the sides of the pat.

Preliminary examination of the data shows distinct seasonal changes in insect activity, as indicated by holes in the tops and bottoms of the pats.

The greatest average loss observed by mid-summer was 52% of the dung on a treatment plot which occurred over approximately 45 days from early June through mid-July, 1979. Field observations indicated that the dry pats were sustaining renewed visitation and consumption in July and August, primarily by grasshoppers. At the time of this report, the data had not been examined for sulfur dioxide treatment effects. Final measurements will be carried out in the EVST Laboratory and the results presented in the Sixth Interim Report, 1980.

TABLE 18.3. MEAN WEIGHTS OF 415 CM^3 PATS OF BOVINE MANURE

Experiment No.	Wet Weight (Grams)					Dry Weight (Grams)					Moisture (Percent)
	n	$\overline{X}$	S.D.	S.E.	C.V.	n	$\overline{X}$	S.D.	S.E.	C.V.	
A79I	8	451.0	8.11	2.87	1.8	7	69.00	3.045	1.151	4.4	84.7
A79II	8	439.1	5.89	2.08	1.3	8	69.36	1.635	0.578	2.4	84.2
A79I&II	16	445.1	9.19	2.30	2.1	15	69.19	2.312	0.597	3.3	84.5
B79I	8	456.0	8.49	3.00	1.9	8	94.30	4.801	1.697	5.1	79.3
B79II	8	447.6	6.37	2.25	1.4	8	93.07	2.611	0.923	2.8	79.2
B79I&II	16	451.8	8.44	2.11	1.9	16	93.69	3.786	1.339	4.0	79.3

Tube Tests of Beetle Movements and Orientation

The initial tube tests at ZAPS produced contradictory results. At times *Canthon laevis* appeared to demonstrate a distinct adversion to sulfur dioxide and at other times just the opposite response was observed. It became apparent that factors such as temperature, slope, wind direction, and orientation to the sun were interacting. Therefore, an intensive study of these factors was initiated before continuing tests of response to sulfur dioxide. These experiments were being carried out at the time of this report. The results will appear at a later time.

The technique seemed to be useful for tests involving *Canthon laevis*. It proved to be of little value with *Nicrophorus* species. Upon release, *Canthon laevis* soon began to move about, walking easily and usually oriented with the axis of the tube. On the other hand, *Nicrophorus* beetles spent most of the test period trying to squeeze through the mesh. Many of the smaller individuals eventually escaped. Movements within the tube by these beetles were extremely limited. Temperatures in excess of 32°C suppressed movement by *Canthon* beetles. At these temperatures the beetles would either remain sedentary at the point of release or would walk until encountering shade from the vegetation and then stop all but very limited movements. At these temperatures, most *Nicrophorus* beetles perished, presumably from heat prostration.

DISCUSSION

As in previous years, the pitfall trapping at both ZAPS I and II demonstrated more captures of *Canthon laevis* on the controls than on any of the sulfur dioxide-treated plots. This difference was significant ($p \leq 0.005$) for total captures, exterior captures, and interior captures. Unlike 1976 and 1977, the population abundance of *C. laevis*, as indicated by pitfall captures, was not similar at ZAPS I and II. There were 2.7 times as many beetles captured at ZAPS I as at ZAPS II. In 1976 at both sites, captures of *C. laevis* were inversely correlated with sulfur dioxide concentrations. In 1977, an inverse correlation was observed for ZAPS II, but at ZAPS I there was a sharp drop-off in numbers on the B plot as compared to the control plot (A plot), and captures on the B, C, and D plots were similar. The 1978 captures at both ZAPS I and II displayed a pattern like that seen at ZAPS I in 1977. Based on the arithmetic mean for sulfur dioxide fumigation levels (Lee *et al.*, 1979), the treatment levels were greater in 1978 than 1977 at both ZAPS--1.4-19.2 pphm at ZAPS I and 1.1-17.8 pphm at ZAPS II versus 1.1-15.3 pphm at ZAPS I and 1.0-16.9 pphm at ZAPS II. This suggests that higher fumigation levels in 1978 may have induced a more pronounced response by the beetles.

Total captures of *Canthon laevis* at both ZAPS I and II exceeded peak population abundance for any previous year. In 1978, rainfall at Colstrip, Montana, exceeded that of 1977 by a factor of 1.48 and that of 1976 by a factor of 1.42. May and June precipitation was 20.22 cm for 1976, 12.04 cm for 1977, and 27.05 cm for 1978 (Munshower, personal communication, 1979). Based on trapping experience at ZAPS, captures increase following rain and are lowest during extended hot, dry periods. From my own observations and frequent comments by other investigators working with invertebrate scavengers of dung and carrion, rainfall extends the period of resource utilization by slowing the drying process and by rehydrating the organic material. Thus, the large number of captures in 1978 may have been due to greater precipitation, which affects beetle activity and may promote population growth by maintaining the suitability of the food resource.

The 1978 pitfall data clearly indicated that sulfur dioxide affected populations of *Canthon* even at the lowest fumigation level. Since the reduction in capture success was similar on all of the treated plots as compared to the controls, it appears that the threshold for the response to sulfur dioxide occurs at or below the lowest fumigation level.

No distinct trends in captures as related to sulfur dioxide for any other species of beetles were noted for 1978, although some other species have been seen to respond to sulfur dioxide in previous years. However, capture numbers of species other than *Canthon* in 1978 were small and the data characterized by high variability. Thus, even if responses occurred, the "noise" in the data would have obscured any trends.

It appears unlikely that sulfur dioxide induced changes in population dynamics such as altered rates of natality or mortality would explain the *Canthon* responses observed at ZAPS. If the insects had a restricted range, this would be an acceptable hypothesis. But the behavioral observations at ZAPS clearly indicate that these beetles are far ranging and capable of traveling considerable distances in a short period of time. Since a beetle released on a plot may be recaptured on any other plot, this indicates a range greater than that of the entire series of plots, and as such, natality or mortality of a resident population could affect total captures at a ZAPS site but not necessarily captures on individual plots.

Whether this behavioral response to sulfur dioxide takes the form of a taxes (directed reactions or movements) or kineses (undirected reactions or movements) remains to be discerned. Anemotaxic, geotaxic, phototaxic, telotaxic, and klinotaxic reactions are being investigated via the tube tests. Observations made at ZAPS in 1979 of *Canthon* arriving at dung on the control plot (A) versus the highest treatment plot (D) suggest an orthokinetic response.

The fecal and carrion decomposition studies clearly demonstrate the speed with which scavenger beetles arrive, the substantial amounts of organic material that is shredded, consumed, and buried, and the increased area of dispersal of this material via activities such as ball rolling. A large and varied dung and carrion community, microseral succession, and competition are obvious characteristics. Although decomposition, nutrient recycling, and soil improvement are obvious benefits, other benefits such as competition for the resource with pest or disease-carrying insects, clean-up on rangelands and reduction of wastage of fouled forage, and immediate recycling of the organic material to food chains via insectivorous animals may be as important if not more so.

Figure 18.7 attempts to model some of these factors. Quantification of the main pathways has not been accomplished to date, but the results of the 1979 field research and incorporation of published information should provide a means for developing a more specific model for inclusion in the final project reports.

Other Observations

A number of behavioral observations for *Canthon laevis* will be covered in the next interim report. A few are of interest with regard to the studies described here.

Dung removal by *Canthon* is accomplished by: (1) feeding, (2) ball rolling, and (3) shredding usually in conjunction with feeding or ball rolling. The distance a dung ball is rolled from the dung pat is variable. Dung balls have been found immediately under the pat, presumably to be buried in the moist soil there. Generally, the balls are rolled away from the immediate proximity of the dropping. For example, in one trial in which 19 dung balls were formed, the average distance of deposition from the edge of the pat (burial or abandonment) was 72.1 ± 52.2 cm. Three balls were left by the edge of the dropping and two were moved twice. Of those moved twice, one was rolled off by two beetles. At a resting point, part of the ball was consumed by the two.

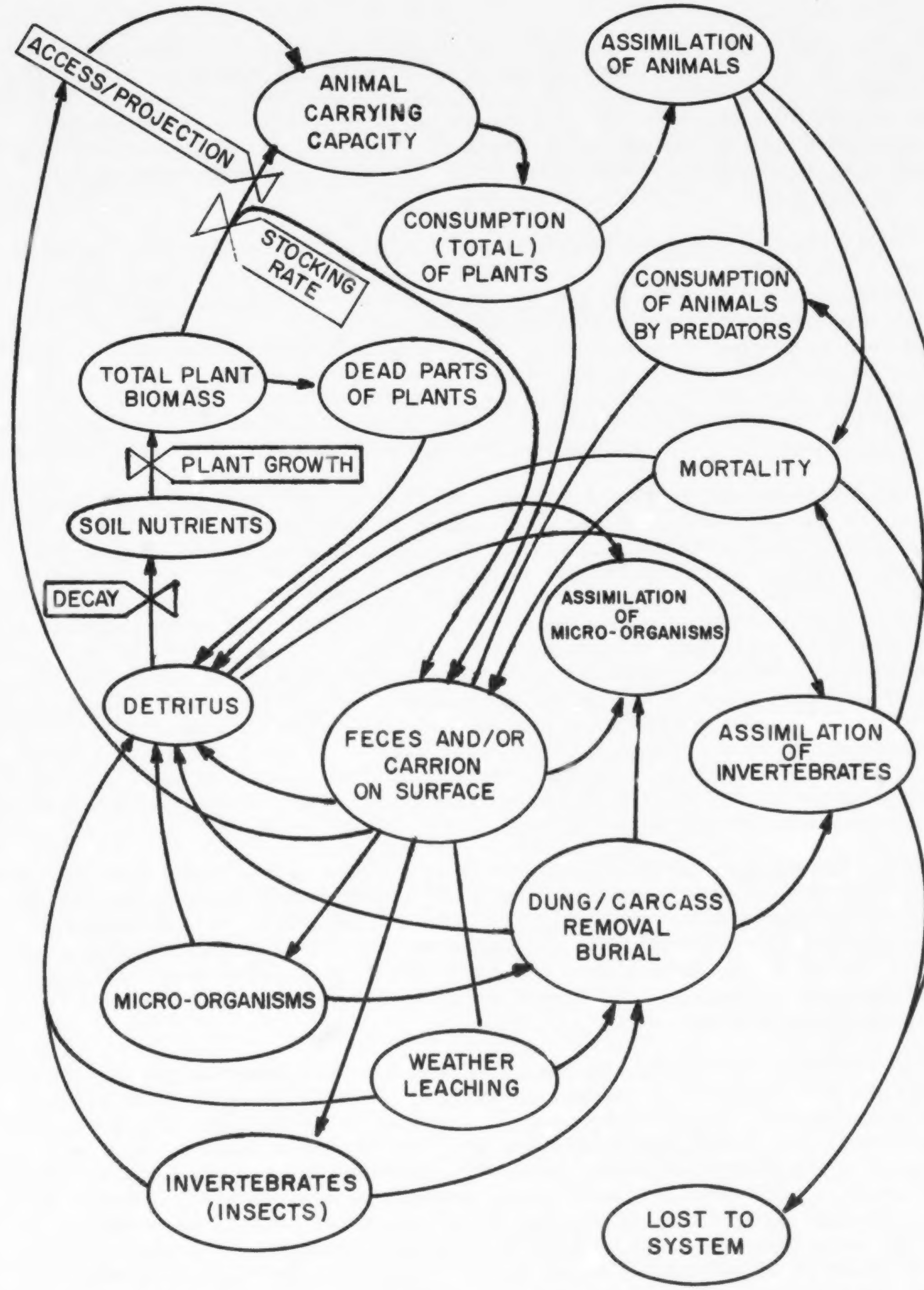
ACCESS/PROJECTION
ANIMAL CARRYING CAPACITY
ASSIMILATION OF ANIMALS
STOCKING RATE
CONSUMPTION (TOTAL) OF PLANTS
CONSUMPTION OF ANIMALS BY PREDATORS
TOTAL PLANT BIOMASS
DEAD PARTS OF PLANTS
PLANT GROWTH
MORTALITY
SOIL NUTRIENTS
DECAY
ASSIMILATION OF MICRO-ORGANISMS
DETRITUS
FECES AND/OR CARRION ON SURFACE
ASSIMILATION OF INVERTEBRATES
DUNG/CARCASS REMOVAL BURIAL
MICRO-ORGANISMS
WEATHER LEACHING
INVERTEBRATES (INSECTS)
LOST TO SYSTEM

Figure 18.7. Interactions of Coprophagous and Necrophagous beetles (based on McKinney and Morley, 1975, and Olechowicz, 1977).

Then, one beetle reformed the remainder of the ball into a smooth sphere and continued to roll it off. In the second case, a beetle abandoned a ball it was rolling. About 30 minutes later either the same beetle or another beetle wandered off of a nearby dung pat pursuing a rather erratic course through the vegetation until coming within sight of the ball, at which time the beetle dashed over to the ball, examined it from all sides, and then continued to roll it off.

Balls were only formed from moist dung. Usually the dung used to build the ball was pulled from the top or sides of the pat. When feeding and shredding, *Canthon* usually worked around the edge of the pat or from underneath. On hot, sunny days with temperatures over 32°C, only feeding and shredding activities were observed. At these times, all of the beetles would either be under the pat or in the shaded area under the edge of the pat opposite the position of the sun. When temperatures were high, newly arrived beetles quickly slid under the edge of the dropping. At times, so many beetles were under a pat that the entire surface undulated almost as if it were pulsing.

Sudden movements by the observer disturbed the beetles, although sounds did not, as far as could be determined. Beetles were particularly likely to cease activities and fly or run off if disturbed soon after arriving at the dung or carcass. When feeding, or building or rolling balls, they seemed to pay little attention to the observer. When disturbed, they either fled by flight, running away, or burrowing rapidly into the dung or under vegetation. If cornered or captured, such as by being picked up by hand, they often "played dead," remaining totally motionless for periods up to several minutes even after being released. When in this state of suspended animation, they can be moved around, picked up, prodded, and still show no indication of being alive and active. The appendages are held rigid. If left alone, they eventually begin to make some very hesitant and limited movements, such as moving a leg or antenna. If not disturbed at this point, they shortly resume activity, usually not leaving the area where they were disturbed.

It is almost impossible to hold a moving beetle. Invariably, the beetle crawls into the space between the base of the fingers and begins digging and prying movements with its powerful prothoracic legs which are broadened and strengthened for burrowing. The sensation is uncomfortable to the holder because the legs are hard, ridged, and have rather sharp tubercles. Even if the discomfort is ignored, the beetle normally succeeds in shoving its way out between the fingers.

The responses of *Canthon laevis* in response to the presence and movements of an observer demonstrate several strategies of self-defense. This points to an evolutionary fitness selection in response to predation. The Australian observations that birds and amphibians prey on these beetles are important in that for the most part these predators rely heavily upon movement to detect their quarry. The cryptic (dull brown, greenish brown, or black) colors of these beetles, in addition to the tactic of remaining motionless would be distinct advantages in escaping notice by these predators. Fleeing and hiding would be of advantage if the predator were too far away to reach the beetle before it could make its escape, or if the predator suddenly encountered the beetle. It is even possible that the digging and prying actions taken by a beetle held in a hand could enable the beetle to wrench itself free of some captors. Swallowing a scrabbling beetle might be less than a pleasant experience and might serve to dissuade the predator from eating any others.

Given the complexity of the insect communities of dung and carrion, the significance of an alteration in the abundance of one or more species is a difficult question. On the one hand, it is possible that any reduction in numbers of beetles such as *Canthon laevis* could be offset by concurrent shifts in other species. Thus, a process such as decomposition might not demonstrate any alteration if a new equilibrium were established. On the other hand, one or more species may serve a unique or critical function. For example, suppression of populations of harmful flies such as flesh flies, horn flies, and disease vectors appears to be a function in which certain beetles play a particularly important role. Although there are a number of predators and parasites of these flies, the dung beetles appear to be particularly efficient competitors for food and space resources in the following ways: (1) removal and consumption of quantities of dung and carrion, (2) transportation of mites that prey on flies, (3) dislodging and breaking up egg masses by feeding and shredding, (4) aeration of the dung and carrion increasing the drying rate, and (5) riddling with entry holes, exit holes, and galleries which provide avenues of access for predators such as staphylinid beetles which do not construct their own corridors.

The use of a standardized bovine dung pat represents an attempt to examine in a more holistic manner an ecological unit and the effects of sulfur dioxide on that unit. The finding that *Canthon laevis* is particularly susceptible to the influence of sulfur dioxide provides an indicator organism that has considerable potential as an early warning mechanism concerning the presence of low levels of sulfur dioxide. An understanding of the mechanism involved should provide a more refined means of utilizing the indicator and should provide some insights into the potential consequences of sulfur dioxide induced reactions both to the indicator organism and to the ecological structural and functional units of which it is a component. Hopefully, approaches such as the use of a standardized dung unit could provide a microcosm for use which would be much more information rich concerning pollution impacts. An obvious disadvantage is interpretation of large amounts of information and separation and identification of the interacting driving variables. At this point, it is impossible to say if dung pats could serve this type of purpose, but there is a need for these types of bioassay tools. Attempts already have been made to develop a soil microcosm test in order to address questions such as: (1) the mobility of a pollutant through the system, (2) the form in which the pollutant is exported from the soil, (3) disruption or alterations of nutrient cycling, (4) effects on soil biota, and (5) dose-responses to the pollutant with respect to mobility, export, nutrient cycling, and population dynamics (Duke *et al.*, 1977).

CONCLUSIONS

1. Beetles of the species *Canthon*, mostly *laevis*, respond to low level sulfur dioxide fumigation. Captures on the control plots (A) were significantly higher than for any of the sulfur dioxide treatment plots at both ZAPS in 1976, 1977, and 1978.

2. The reaction of *Canthon laevis* to sulfur dioxide appears to be primarily behavioral and not due to altered life table factors such as changes in natality or mortality.

3. Whether the response is a kinesis or taxis or both has not been determined, but preliminary evidence suggests an orthokinesis.

4. Scavenger beetles make significant contributions to the rapid breakdown and removal of dung and carrion in rangelands.

5. These scavenging activities contribute not only to the quick turnover of nutrients but also result in the cleaning up of fouled herbage and strong competition for food and space resources with pest insects such as parasites and carriers of disease.

6. *Canthon laevis* employs several defensive strategies when disturbed, in addition to being cryptically colored. This indicates that they either are or have been a food resource for some other organism(s).

7. The beetle studies at ZAPS have not only revealed information concerning the responses to sulfur dioxide but have provided a considerable amount of information concerning the biology of these scavengers or decomposers in grasslands of the Northern Great Plains.

REFERENCES

Allee, W. C., A. E. Emerson, O. Park, T. Park, and K. P. Schmidt. 1949. Principles of Animal Ecology. W. B. Saunders Co., Philadelphia, Pa. 837 pp.

Andrzejewska, L. 1974. Analysis of a Sheep Pasture Ecosystem in the Pieniny Mountains (The Carpathians). V. Herbivores and Their Effect on Plant Production. Ekol. Pol., 22:527-534.

Blume, R. R. 1970. Insects Associated with Bovine Droppings in Kerr and Becar Counties. Texas J. Econ. Entomol., 63:1023-1024.

Blume, R. R., S. E. Kunz, B. F. Hogan, and J. J. Matter. 1970. Biological and Ecological Investigations of Horn Flies in Central Texas: Influences of Other Insects in Cattle Manure. J. Econ. Entomol., 63:1121-1123.

Bromenshenk, J. J. 1978. Investigation of the Impact of Coal-Fired Power Plant Emissions Upon Insects: Entomological Studies at the Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Or. pp. 473-507.

Bromenshenk, J. J. 1979. Responses of Ground-Dwelling Insects to Sulfur Dioxide. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 673-722.

Brown, W. J. 1940. Notes on the American Distribution of Some Species of Coleoptera Common to the European and North American Continents. Can. Entomol., 72(4):70-72.

Coffey, M. D. 1966. Studies on the Association of Flies (Diptera) with Dung in Southeastern Washington. Ann. Entomol. Soc. Am., 59(1):207-217.

Duke, K. M., M. E. Davis, and A. J. Dennis. 1977. IERL-RTP Procedures Manual: Level 1 Environmental Assessment Biological Tests for Pilot Studies. EPA-600/7-77-043, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 105 pp.

Durie, P. 1975. Some Possible Effects of Dung Beetle Activity on the Infestation of Pastures by Intestinal Worm Larvae of Cattle. J. Appl. Ecol., 12(3):831.

Gillard, P. 1967. Coprophagous Beetles in Pasture Ecosystems. J. Aust. Inst. Agr. Sci., 33:3-34.

Green, R. 1966. Gut Contents of Some Tasmanian Birds. The EMU, 66:105-110.

Hammer, O. 1941. Biological and Ecological Observations on Flies Associated with Pasturing Cattle and Their Excrement. Videsk. Medd. Dansk. Naturhist. Foren. Kjobenhaun, 105:141-393.

Harris, R. L., and L. M. Oliver. 1979. Predation of *Philonthus flavolimbatus* on the Horn Fly. Environ. Entomol., 8:250-260.

Hayes, W. P. 1927. Prairie Insects. Ecology, 8:238-250.

Hayes. W. P. 1929. Morphology, Taxonomy, and Biology of Larval Scarabaeoides. Ill. Biol. Mono., 12:1-119.

Kulman, H. M. 1974. Comparative Ecology of North American Carabidae with Special Reference to Biological Control. Entomophaga Mem. H. S., 7:61-70.

Lee, J. J., E. M. Preston, and D. Weber. 1979. Temporal Variation in Sulfur Dioxide Concentration on ZAPS. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 284-305.

Leetham. J. W., T. J. McNary, and J. L. Dodd. 1979. Effects of Controlled Levels of Sulfur Dioxide on Invertebrate Consumers. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 723-763.

MacQueen, A. 1975. Dung as an Insect Food Source: Dung Beetles as Competitors of Other Coprophagous Fauna and as Targets for Predators. J. Appl. Ecol., 12(3):821-827.

MacQueen, A., and R. P. Beirne. 1975. Influence of Other Insects on Production of Horn Fly, *Haematobia irritans* (Diptera: Muscidae), From Cattle Dung in South-Central British Columbia. Can Entomol., 107:1255-1264.

McKinney, G. T., and F. H. W. Morley. 1975. The Agronomic Role of Introduced Dung Beetles in Grazing Systems. J. Appl. Ecol., 12(3):831-836.

Mohr, C. O. 1943. Cattle Droppings as Ecological Units. Ecol. Mono., 13(3):275-298.

Morrill, W. L. 1975. Plastic Pitfall Trap. Environ. Entomol., 4:596.

Olechowicz, E. 1974. Analysis of a Sheep Pasture Ecosystem in the Pieniny Mountains (The Carpathians). X. Sheep Dung and the Fauna Colonizing It. Ekol. Pol., 22:589-616.

Olechowicz, E. 1976. The Role of Coprophagous Dipterans in a Mountain Pasture Ecosystem. Ekol. Pol., 24:124-165.

Olechowicz, E. 1977. The Effect of Coprophages on Feces Decomposition in a Pasture Ecosystem. Ecol. Bull. (Stockholm), 25:553-556.

Olson, Elliott, and Associates. 1976. Effects of Spruce Budworm Control on Pollinating Insects. USDA Forest Service, Region 1, Missoula, Mt. 56 pp.

Payne, J. A. 1965. A Summer Carrion Study of the Baby Pig *Sus scrofa* Linnaeus. Ecology, 46(5):592-602.

Portchinsky, I. A. 1885. Muscarum Cadaverinarum Stercoriarumque Biologia Comparata. Horae Soc. Entomol. Ross., 19:210-244.

Preston, E. M., and T. Gullett. 1979. Spatial Variation of Sulfur Dioxide Concentrations on ZAPS during the 1977 Field Season. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, E. M. Preston and T. L. Gullett, eds. EPA-600/3-79-044, U.S. Environmental Protection Agency, Corvallis, Or. pp. 306-330.

Rowley, I., and W. J. M. Vestjens. 1973. The Comparative Ecology of Australian Corvids. V. Food. Aust. Wildl. Res., 18:131-156.

Sanders, D. P., and R. C. Dobson. 1966. The Insect Complex Associated with Bovine Manure in Indiana. Ann. Entomol. Soc. Am., 59(5):955-959.

Shubeck, P. P. 1968. Oreientation of Carrion Beetles to Carrion: Random or Non-random? J. New York Entomol. Soc., 76(4):253-265.

Shubeck, P. P. 1976. An Alternative to Pitfall Traps in Carrion Beetle Studies (Coleoptera). Entomol. News, 87(5-6):176-178.

Southwood, T. R. E. 1975. Ecological Methods with Particular Reference to the Study of Insect Populations. Chapman and Hall Ltd., London. 391 pp.

Thomas, D. B., Jr., and E. L. Sleeper. 1977. The Use of Pitfall Traps for Estimating the Abundance of Arthropods, with Special Reference to the Tenebrionidae (Coleoptera). Ann. Entomol. Soc. Am., 70(2):242-248.

Thomas, G. D. 1967. Natural Enemies of the Face Fly *Musca autumnalis* De Geer in Missouri. Ph.D. Thesis, University of Missouri, Columbia, Mo. 131 pp.

Thomas, G. D., and C. E. Morgan. 1972. Field-Mortality Studies of the Immature Stages of the Horn Fly in Missouri. Environ. Entomol., 1:453-459.

Vestjens, W. J. M., and R. Carrick. 1974. Food of the Black-Backed Magpie, *Gymnorhina* T, Tibicen, at Canberra. Aust. Wildl. Res., 1:71-83.

APPENDIX TABLE 18.1. 7 X 7 GRID CAPTURE OF *CANTHON* SP, AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 20,456
Trap Days - 196
June 30 - July 10, 1978

PLOT A (Control)

	Total Traps	Perimeter Traps	Interior Traps
Σ	7,001	4,777	2,224
$\bar{X}$	142.9	199.0	89.0
S	120.4	120.2	94.7
$S_{\bar{x}}$	17.2	24.5	18.9

PLOT B (Low)

June 30 - July 10, 1978

	Total Traps	Perimeter Traps	Interior Traps
Σ	4,787	3,426	1,361
$\bar{X}$	97.7	142.8	54.5
S	89.4	94.2	59.0
$S_{\bar{x}}$	12.8	19.2	11.8

PLOT C (Medium)

June 30 - July 10, 1978

	Total Traps	Perimeter Traps	Interior Traps
Σ	4,242	2,744	1,498
$\bar{X}$	86.6	114.3	59.9
S	102.0	115.9	80.0
$S_{\bar{x}}$	14.6	23.7	16.0

PLOT D (High)

June 30 - July 10, 1978

	Total Traps	Perimeter Traps	Interior Traps
Σ	4,426	3,005	1,421
$\bar{X}$	90.3	125.2	56.8
S	75.7	80.2	54.1
$S_{\bar{x}}$	10.456	16.4	10.8

ZAPS II

Grand Total - 7,620
Trap Days - 196
June 30 - July 10, 1978

PLOT A (Control)

	Total Traps	Perimeter Traps	Interior Traps
Σ	2,887	2,264	623
$\bar{X}$	58.9	94.3	24.9
S	86.6	99.6	54.9
$S_{\bar{x}}$	12.4	20.3	11.0

PLOT B (Low)

June 30 - July 10, 1978

	Total Traps	Perimeter Traps	Interior Traps
Σ	1,515	1,425	90
$\bar{X}$	30.9	59.4	3.6
S	63.2	81.3	8.2
$S_{\bar{x}}$	9.0	16.6	1.6

PLOT C (Medium)

June 30 - July 10, 1978

	Total Traps	Perimeter Traps	Interior Traps
Σ	1,010	776	234
$\bar{X}$	20.6	32.3	9.4
S	31.1	38.8	14.9
$S_{\bar{x}}$	4.4	7.9	3.0

PLOT D (High)

June 30 - July 10, 1978

	Total Traps	Perimeter Traps	Interior Traps
Σ	1,808	1,658	150
$\bar{X}$	36.9	69.1	6.0
S	56.3	65.7	13.1
$S_{\bar{x}}$	8.0	13.4	2.6

APPENDIX TABLE 18.2. 7 X 7 GRID CAPTURE OF *NICROPHORUS* SP. AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 349
Trap Days - 196
June 30 - July 10, 1978

PLOT A (Control)

Total Traps	Perimeter Traps	Interior Traps
Σ 83	Σ 51	Σ 32
$\bar{X}$ 1.7	$\bar{X}$ 2.1	$\bar{X}$ 1.3
S 2.4	S 2.8	S 2.0
$S_{\bar{x}}$ 0.4	$S_{\bar{x}}$ 0.6	$S_{\bar{x}}$ 0.4

PLOT B (Low) June 30 - July 10, 1978

Total Traps	Perimeter Traps	Interior Traps
Σ 83	Σ 47	Σ 36
$\bar{X}$ 1.7	$\bar{X}$ 2.0	$\bar{X}$ 1.4
S 2.0	S 2.5	S 1.5
$S_{\bar{x}}$ 2.3	$S_{\bar{x}}$ 0.5	$S_{\bar{x}}$ 0.3

PLOT C (Medium) June 30 - July 10, 1978

Total Traps	Perimeter Traps	Interior Traps
Σ 125	Σ 31	Σ 94
$\bar{X}$ 2.6	$\bar{X}$ 1.3	$\bar{X}$ 3.8
S 8.4	S 1.5	S 11.7
$S_{\bar{x}}$ 1.2	$S_{\bar{x}}$ .3	$S_{\bar{x}}$ 2.3

PLOT D (High) June 30 - July 10, 1978

Total Traps	Perimeter Traps	Interior Traps
Σ 58	Σ 35	Σ 23
$\bar{X}$ 1.2	$\bar{X}$ 1.5	$\bar{X}$ 0.9
S 1.9	S 2.1	S 1.5
$S_{\bar{x}}$ 0.3	$S_{\bar{x}}$ 0.4	$S_{\bar{x}}$ 0.3

ZAPS II

Grand Total - 616
Trap Days - 196
June 30 - July 10, 1978

PLOT A (Control)

Total Traps	Perimeter Traps	Interior Traps
Σ 152	Σ 55	Σ 97
$\bar{X}$ 3.1	$\bar{X}$ 2.3	$\bar{X}$ 3.9
S 3.7	S 2.7	S 4.4
$S_{\bar{x}}$ 0.5	$S_{\bar{x}}$ 0.6	$S_{\bar{x}}$ 0.9

PLOT B (Low) June 30 - July 10, 1978

Total Traps	Perimeter Traps	Interior Traps
Σ 139	Σ 82	Σ 57
$\bar{X}$ 2.8	$\bar{X}$ 3.4	$\bar{X}$ 2.3
S 3.0	S 3.6	S 2.2
$S_{\bar{x}}$ 0.4	$S_{\bar{x}}$ 0.7	$S_{\bar{x}}$ 0.5

PLOT C (Medium) June 30 - July 10, 1978

Total Traps	Perimeter Traps	Interior Traps
Σ 117	Σ 82	Σ 35
$\bar{X}$ 2.4	$\bar{X}$ 3.4	$\bar{X}$ 1.4
S 3.0	S 3.7	S 1.8
$S_{\bar{x}}$ 0.4	$S_{\bar{x}}$ 0.8	$S_{\bar{x}}$ 0.4

PLOT D (High) June 30 - July 10, 1978

Total Traps	Perimeter Traps	Interior Traps
Σ 208	Σ 153	Σ 55
$\bar{X}$ 4.2	$\bar{X}$ 6.4	$\bar{X}$ 2.2
S 4.3	S 4.9	S 2.0
$S_{\bar{x}}$ 0.6	$S_{\bar{x}}$ 1.0	$S_{\bar{x}}$ 0.4

APPENDIX TABLE 18.3. 7 X 7 GRID CAPTURE OF *PHANAEUS* SP. AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 49
Trap Days - 196
June 30 - July 10, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 18	Σ 7	Σ 14	Σ 10
$\bar{X}$ 0.37	$\bar{X}$ 0.14	$\bar{X}$ 1.56	$\bar{X}$ 1.11
S 0.07	S 0.41	S 1.13	S 0.33
$S_{\bar{x}}$ 0.10	$S_{\bar{x}}$ 0.06	$S_{\bar{x}}$ 0.16	$S_{\bar{x}}$ 0.05

ZAPS II

Grand Total - 17
Trap Days - 196
June 23 - July 3, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 5	Σ 2	Σ 5	Σ 5
$\bar{X}$ 0.10	$\bar{X}$ 0.04	$\bar{X}$ 0.10	$\bar{X}$ 0.10
S 0.31	S 0.29	S 0.37	S 0.37
$S_{\bar{x}}$ 0.04	$S_{\bar{x}}$ 0.04	$S_{\bar{x}}$ 0.05	$S_{\bar{x}}$ 0.05

APPENDIX TABLE 18.4. 7 X 7 GRID CAPTURE OF *ONTHOPHAGUS* SP. AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 18
Trap Days - 192
June 30 - July 10, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 4	Σ 11	Σ 2	Σ 1
$\bar{X}$ 0.08	$\bar{X}$ 0.22	$\bar{X}$ 0.04	$\bar{X}$ 0.02
S 0.28	S 0.55	S -.29	S 0.14
$S_{\bar{x}}$ 0.04	$S_{\bar{x}}$ 0.08	$S_{\bar{x}}$ 0.04	$S_{\bar{x}}$ 0.02

ZAPS II

Grand Total - 8
Trap Days - 192
June 23 - July 3, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 1	Σ 2	Σ 1	Σ 4
$\bar{X}$ 0.02	$\bar{X}$ 0.04	$\bar{X}$ 0.02	$\bar{X}$ 0.08
S 0.14	S 0.29	S 0.14	S 0.28
$S_{\bar{x}}$ 0.02	$S_{\bar{x}}$ 0.04	$S_{\bar{x}}$ 0.02	$S_{\bar{x}}$ 0.04

APPENDIX TABLE 18.5. 7 X 7 GRID CAPTURE OF *PASIMACHUS* SP. AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 56
Trap Days - 196
June 30 - July 10, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 5	Σ 19	Σ 14	Σ 18
$\bar{X}$ 0.10	$\bar{X}$ 0.39	$\bar{X}$ 0.29	$\bar{X}$ 0.37
S 0.47	S 0.76	S 0.58	S 0.81
$S_{\bar{x}}$ 0.07	$S_{\bar{x}}$ 0.11	$S_{\bar{x}}$ 0.08	$S_{\bar{x}}$ 0.12

ZAPS II

Grand Total - 24
Trap Days - 196
June 30 - July 10, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 1	Σ 8	Σ 8	Σ 7
$\bar{X}$ 0.02	$\bar{X}$ 0.16	$\bar{X}$ 0.16	$\bar{X}$ 0.14
S 0.14	S 0.43	S 0.37	S 0.41
$S_{\bar{x}}$ 0.02	$S_{\bar{x}}$ 0.06	$S_{\bar{x}}$ 0.05	$S_{\bar{x}}$ 0.06

APPENDIX TABLE 18.6. 7 X 7 GRID CAPTURE OF *CINCINDELLA* SP. AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 21
Trap Days - 196
June 30 - July 10, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 12	Σ 0	Σ 0	Σ 9
$\bar{X}$ 0.24	$\bar{X}$ -	$\bar{X}$ -	$\bar{X}$ 0.18
S 0.60	S -	S -	S 0.57
$S_{\bar{x}}$ 0.09	$S_{\bar{x}}$ -	$S_{\bar{x}}$ -	$S_{\bar{x}}$ 0.08

ZAPS II

Grand Total - 7
Trap Days - 196
June 30 - July 10, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 3	Σ 2	Σ 1	Σ 1
$\bar{X}$ 0.06	$\bar{X}$ 0.04	$\bar{X}$ 0.02	$\bar{X}$ 0.02
S 0.32	S 0.29	S 0.14	S 0.14
$S_{\bar{x}}$ 0.05	$S_{\bar{x}}$ 0.04	$S_{\bar{x}}$ 0.02	$S_{\bar{x}}$ 0.02

APPENDIX TABLE 18.7. 7 X 7 GRID CAPTURE OF WEEVIL SP. AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 2
Trap Days - 196
June 30 - July 10, 1978

Total Traps

	PLOT A	PLOT B	PLOT C	PLOT D
Σ	0	0	1	1
$\bar{X}$	-	-	0.02	0.02
S	-	-	0.14	0.14
$S_{\bar{x}}$	-	-	0.02	0.02

ZAPS II

Grand Total - 8
Trap Days - 196
June 30 - July 10, 1978

Total Traps

	PLOT A	PLOT B	PLOT C	PLOT D
Σ	3	1	0	4
$\bar{X}$	0.06	0.02	0	0.08
S	0.32	0.14	-	0.34
$S_{\bar{x}}$	0.05	0.02	-	0.05

APPENDIX TABLE 18.8. 7 X 7 GRID CAPTURE OF *TROX* SP. AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 59
Trap Days - 196
June 30 - July 10, 1978

Total Traps

	PLOT A	PLOT B	PLOT C	PLOT D
Σ	6	16	16	21
$\bar{X}$	0.12	0.33	0.33	0.43
S	0.44	0.59	0.75	0.71
$S_{\bar{x}}$	0.06	0.08	0.11	0.10

ZAPS II

Grand Total - 98
Trap Days - 196
June 30 - July 10, 1978

Total Traps

	PLOT A	PLOT B	PLOT C	PLOT D
Σ	13	36	28	21
$\bar{X}$	0.27	0.73	0.57	0.43
S	0.64	1.00	0.94	0.76
$S_{\bar{x}}$	0.09	0.14	0.13	0.11

APPENDIX TABLE 18.9. 7 X 7 GRID CAPTURE OF OTHER BEETLES AT ZAPS I AND II, 1978

ZAPS I

Grand Total - 293
Trap Days - 196
June 30 - July 10, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 36	Σ 97	Σ 121	Σ 39
$\bar{X}$ 0.73	$\bar{X}$ 1.98	$\bar{X}$ 2.47	$\bar{X}$ 0.80
S 1.24	S 2.02	S 2.20	S 1.32
$S_{\bar{x}}$ 0.18	$S_{\bar{x}}$ 0.29	$S_{\bar{x}}$ 0.31	$S_{\bar{x}}$ 0.19

ZAPS II

Grand Total - 220
Trap Days - 196
June 30 - July 10, 1978

Total Traps

PLOT A	PLOT B	PLOT C	PLOT D
Σ 34	Σ 82	Σ 48	Σ 56
$\bar{X}$ 0.69	$\bar{X}$ 1.67	$\bar{X}$ 0.98	$\bar{X}$ 1.14
S 1.23	S 1.66	S 1.07	S 1.87
$S_{\bar{x}}$ 0.18	$S_{\bar{x}}$ 0.24	$S_{\bar{x}}$ 0.15	$S_{\bar{x}}$ 0.27

SECTION 19

RESPONSE OF FIELD POPULATIONS OF TARDIGRADES TO VARIOUS LEVELS OF CHRONIC, LOW-LEVEL SULFUR DIOXIDE EXPOSURE

J. W. Leetham, T. J. McNary, J. L. Dodd, and W. K. Lauenroth

ABSTRACT

The soil populations of Tardigrades of a northern mixed grass prairie were sampled after having been exposed to various low levels of sulfur dioxide. The populations of ZAPS I and ZAPS II sites showed substantial reduction, although it was difficult to show the reductions were significant, due to high variability of sample counts. Substantial reduction in the frequency of occurrence in the samples helped to substantiate the population decreases. Three genera of tardigrades were identified from sample material: *Macrobiotus*, *Hexapodibuis*, and *Diphascon*.

INTRODUCTION

Tardigrades are small (<1.0 mm) arthropod-like animals commonly called "water bears" because most known species are aquatic or semiaquatic. Because their phylogeny is unclear (Riggin 1962), they have been classified with the arthropods by some and given separate phylum status by others. Their functional or trophic status is also unknown, although population estimates in some soil systems range as high as $300,000 \cdot m^{-2}$ (Franz 1941, 1950; Ramazzotti 1959). Tardigrades are often overlooked in soil faunal studies because most commonly used extraction methods are inefficient in retrieving them. Although they may be comparatively less important to ecosystem function than say nematodes, tardigrades may be very sensitive indicators of air pollution effects on soil ecosystem processes.

MATERIALS AND METHODS

Each treatment plot of the ZAPS I and II sites was divided into replicate halves and each of the replicates was subdivided into a grid of sampling locations. A random numbers table was used to choose a set of sampling locations at each of three sampling dates. In July, 1977 and July, 1978, five samples were taken from each replicate of each treatment in both ZAPS I and ZAPS II. In September, 1978, 10 samples were collected from each replicate in ZAPS I only. A sample consisted of a 4.8 cm diameter by 10.0 cm deep soil core from which the tardigrades were extracted by a Baermann funnel process, a commonly used live extraction technique. Specimens were preserved in 70% ethanol and representatives were sent out for identification.

RESULTS AND DISCUSSION

Three tardigrade genera were identified including *Macrobiotus* Schultze, *Hexapodibius*, and *Diphascon* Plate (=*Hypsibius* Ehrenberg). The *Diphascon* is probably undescribed and further work is needed to determine if the others fit recognized species. The mean counts for all sample dates showed substantially reduced populations in the high treatment plots and variable population reductions in the intermediate treatment plots (Figures 19.1 and 19.2). The raw and log-transformed data [log (X+1)] were evaluated with a repeated measures ANOVA. The log transformation seemed appropriate because sample variability tended to increase with the mean and many zero counts occurred in high treatment plots. Because of high variability in the data, significant treatment differences were not found on all dates. For the July dates on ZAPS I, the high treatment plot had significantly lower tardigrade populations than the control plot ($P=.05$) for the raw data only. All other treatment comparisons were not significantly different ($P\leq.10$). No significant treatment differences were found in either the raw or transformed data from the September, 1978 ZAPS I sampling ($P\leq .10$). For the July dates on ZAPS II, a significant treatment effect was found in comparing the control and high treatment raw data ($P=.03$) and transformed data ($P=.09$).

Considering the frequency of occurrence of the tardigrades in the samples, there were substantial reductions with increased SO_2 concentration on all dates on both sites (Table 19.1). The average reduction was greater on ZAPS I (90% to 16.7) than on ZAPS II (60% to 15%).

A majority of the tardigrade specimens that were identified was of the genus *Macrobiotus*. However, since only total counts were made in each sample, no attempt was made to determine SO_2 treatment effect by genus. Whether or not there is differential sensitivity among the three identified genera remains unknown.

We could not show significantly reduced tardigrade populations on the high treatment plots on all dates and ZAPS sites because of high sample variability. However, since some significant differences were found and there was a substantial reduction in frequency of occurrence in the samples in the high treatment plots on all dates, we are confident that the tardigrade populations were reduced by the SO_2 exposure. The high sample variability was probably the result of clumped spatial distribution in tardigrades and the small sample core size (18.1 cm^2 surface area). From recent sampling (1979 season) the tardigrades have been shown to be restricted to the surface 1-2 cm of the soil profile. Additional sampling with larger diameter cores has been done in an attempt to confirm the effect of SO_2 that is apparently reducing the tardigrade populations.

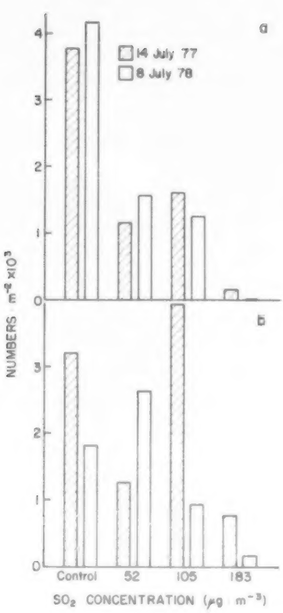

Figure 19.1. Mean counts of Tardigrades on two field sites in southeastern Montana in 1977 and 1978 (a = Site I and b = Site II).

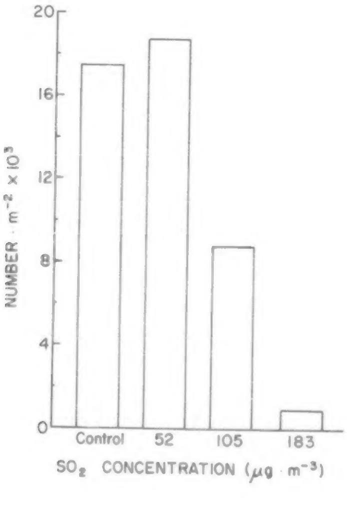

Figure 19.2. Mean counts of Tardigrades on Site I in September, 1978 in southeastern Montana.

TABLE 19.1. FREQUENCY OF OCCURRENCE OF TARDIGRADES IN SAMPLES

	Treatments			
Dates	Control	Low	Medium	High
		Site I		
14 July 77	80	70	60	20
8 July 78	100	90	30	0
16 Sept. 78	90	75	80	30
$\bar{X}$	90	78.33	56.67	16.67
		Site II		
14 July 77	60	20	30	20
8 July 78	60	30	20	10
$\bar{X}$	60	25	25	15
		Total		
$\bar{X}$	78.0	57.0	44.0	16.00
S.E.	8.00	13.56	11.22	5.10

CONCLUSIONS

Since the tardigrades reside on the soil surface, they may, as a group, be more vulnerable to SO_2 and other air pollutants than nematodes and microarthropods which are distributed deeper into the soil profile.

REFERENCES

Franz, H. 1941. Unterschungen über die Bodenbiologie alpiner Gründland und Ackerböden. Forschungsdienst, 11:355-368.

Franz, H. 1950. État de nos connaissances sur da microfaune du sol. Ecologie (Colloques int. cent. natn. Rech. scient., Paris 1950), pp. 81-92.

Ramazzotti, G. 1959. Tardigradi in terreni prativi. Atti. Soc. ital. Sci. nat., 98:199-210.

Riggin, G. T., Jr. 1962. Tardigrada of Southwest Virginia: With the Addition of a Description of a New Marine Species from Florida. Va. Agric. Exp. Stn. Tech. Bull., 152. 145 pp.

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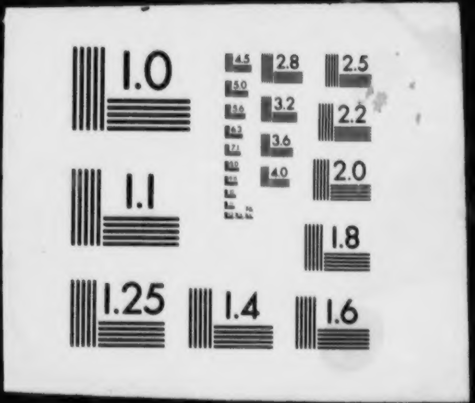
1.0
4.5
2.8
2.5
5.0
3.2
5.6
2.2
6.3
3.6
7.1
8.0
4.0
2.0
1.1
1.8
1.25
1.4
1.6

SECTION 20

RESPONSE OF RANGELAND GRASSHOPPER POPULATIONS TO CONTROLLED LEVELS OF SO_2

T. J. McNary, J. W. Leetham, W. K. Lauenroth, and J. L. Dodd

ABSTRACT

The grasshoppers (Acrididae) on two mixed-grass prairie sites in southeastern Montana were subjected to low-level chronic fumigation with SO_2. A greater reduction in the grasshopper population occurred with greater exposure time and with higher SO_2 concentrations. Possible mechanisms for this population reduction are discussed.

INTRODUCTION

Past studies show that arthropods respond to air pollution in different ways. They adapt by industrial melanism (Kettlewell, 1955, 1956, 1961; Creed, 1975; Bishop *et al.*, 1975; and Lees *et al.*, 1973) and/or they modify their behavior by avoiding the pollution (Bromenshenk and Gordon, 1978). Population changes may occur caused by direct pollution toxicity to the insects (Kanaga, 1956; Hillman and Benton, 1972; and Freitag *et al.*, 1973a).

The purpose of this study is to determine the effects of controlled SO_2 exposure on grasshopper population dynamics and species composition in the mixed-grass prairie of Montana. Grasshoppers, being herbivores, play an important role in energy transfer and nutrient cycling.

MATERIALS AND METHODS

The grasshopper population was estimated by a flush census (Pfadt, 1977). The grasshoppers were flushed by manually brushing the vegetation on a *ca.* $0.1 \cdot m^{-2}$ quadrat. Twenty-five temporary quadrats were systematically located 3-4 m apart along a transect across the treatments. Species and age classification (instar) of each individual was noted. Size, shape, color patterns, songs, and behavior were used for field identification. Collected voucher specimens were verified by Dr. Robert Pfadt (University of Wyoming). Instar identifications were based on size of nymph (Brusven, 1967, 1972; Scoggan and

Brusven, 1972). Sex of each individual was recorded when possible. Species that were not positively identified were grouped in the lowest taxonomic level in the summary.

Censusing was performed at the ZAPS sites in southeast Montana on 10 May, 24 May, 7 June, 15 June, 24 June, 7 July, 22 July, and 7 August in 1977. In 1978 the censusing dates were 26 May, 4 June, 14 June, 1 July, 7 July, 26 July, 3 August, and 17 August. Population estimates were made by replicate for each sample date. In 1977 and the first three dates in 1978, the population estimates for a replicate were based on one transect per replicate. To obtain a more precise estimate of population, two transects were followed on each replicate on 1 July 1978 and thereafter. The analysis of variance used was for repeated measures on a randomized block.

RESULTS AND DISCUSSION

Melanoplus sanguinipes (Fab) was the most common grasshopper found on both sites in 1977 and 1978. This species overwinters as eggs and matures by August. The most common spring maturing species was *Eritettix simplex* (Scudder). Fourteen other species were observed with fewer species observed in 1978 than 1977 (Table 20.1).

Analysis of variance of the total grasshopper population and also that of *M. sanguinipes* showed a significant interaction of treatment x date within each year ($p < .01$). Early in the season the population decreased as the overwintering species died. Then hatching caused the population to rise rapidly. This rise was faster on the control and low treatments than on the high treatments in 1977 and 1978. This results in lower mid and late season populations in the higher treatments (Figure 20.1b and 20.2b).

A significant interaction also occurred in the analysis of site x date within year ($p < 0.01$) for total and the *M. sanguinipes* population. In 1977, at both sites, both the total population (Figure 20.1a) and that of *M. sanguinipes* (Figure 20.2a) start at similar levels, but the Site II population increases faster to a higher level than Site I, before the population declines in the fall. Differences between years occur mainly as a result of hatching being delayed in 1978 because of the wet, cold spring. Here the populations on the two sites are not significantly different until later in the season.

The trend on each site shows decreased population with increased concentration of SO_2. This was even more noticeable in 1978 than in 1977. There were significantly less grasshoppers on the medium and high treatments than on the control on Site II in 1977, and on both sites in 1978 for the medium and high treatments ($p = 0.0672$). These differences also occur in the *M. sanguinipes* population for the medium and high treatments on Site II in 1977, and for all fumigated treatments on Site II in 1978 ($p = 0.0457$).

We can only speculate on how the SO_2 reduced the populations. Increased emigration and decreased immigration rates may be the cause. *Canthon* spp.

TABLE 20.1. PERCENT SPECIES COMPOSITION OF GRASSHOPPERS ON .52 HA TREATMENTS FUMIGATED WITH DIFFERENT LEVELS OF SO_2

	SO_2 Fumigation Level															
	Site I								Site II							
	Control		Low		Medium		High		Control		Low		Medium		High	
Grasshopper species	1977	1978	1977	1978	1977	1978	1977	1978	1977	1978	1977	1978	1977	1978	1977	1978
Aeropedellus clavatus (Thomas)		0.8			2.7				0.8		0.8				3.2	
Ageneotettix deorum (Scudder)	5.0	11.5	9.4	11.8	2.7	11.8	6.8	18.9	4.1		8.9	3.0	1.2	5.3	6.4	
Amphitornus coloradus (McNeill)		1.6							0.8						1.1	
Aulocara elliotti (Thomas)	1.2												1.2			
Drepanopterna femoratum (Scudder)									0.8		0.8					
Eritettix simplex (Scudder)	7.5	18.8	10.8	22.6	8.1	23.6	5.1	16.2	1.6	7.9	0.8	13.1	4.9	16.0	5.3	20.0
Opeia obscura (Thomas)	2.5		2.7				1.7		1.6		3.2		4.9	2.6	6.4	
Unidentified Gomphocerinae	3.8	8.2	9.4	9.7	6.8	10.5	5.1	1.3	6.5	2.1	2.4	3.0	4.9	6.7	9.6	4.0
Melanoplus confusus (Scudder)	1.2			4.3	1.4	2.6		4.0	1.6		1.6				2.1	
M. infantilis (Scudder)	2.5	4.1	1.4	3.2		1.3	1.7	2.7						1.3		
M. packardii (Scudder)	3.8		2.7				1.7				1.6		1.2			6.7
M. sanguinipes (Fabricius)	57.5	36.9	47.3	33.3	60.8	38.1	62.7	36.5	65.0	74.1	67.7	59.6	61.0	49.3	55.3	53.3
Phoetaliotes nebrascensis (Thomas)		3.3	2.7	3.2	2.7	2.6		2.7		4.3	0.8	12.1	3.6	6.7	1.1	10.7
Unidentified Crytacanthacridinae		4.1		3.2	4.0		6.8		2.4	0.7	3.2	1.0		4.0		1.3
Arphia pseudonietana (Thomas)	1.2		1.4		2.7						0.8					
Spharagemon equale (Say)	1.2	0.8	1.4		1.4			2.7	0.8	0.7	3.2	3.0				
Trachyrhachys kiowa (Thomas)	1.2	1.6									0.8					
Xanthippus corallipes (Haldeman)											0.8					
Unidentified Oedipodinae		0.8			2.7		1.7		0.8				1.2			
Unidentified Acrididae	11.2	7.4	10.8	8.6	4.0	9.2	6.8	14.9	13.0	10.7	2.4	5.0	15.8	8.0	9.6	4.0
Actual number observed*	80.0	61.0	74.0	46.5	74.0	38.0	59.0	37.0	123.0	69.5	124.0	49.5	82.0	37.5	94.0	37.5

*Based on 16 transects (25, ca. 0.1 m^2, quadrats per transect) May through August

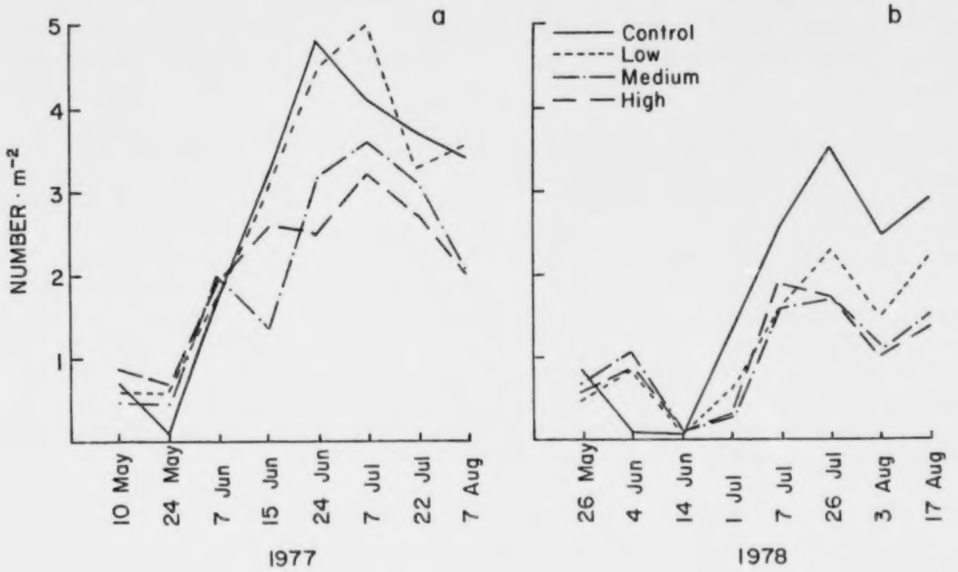

Figure 20.1. Seasonal dynamics of total population of grasshoppers fumigated with four different levels of SO_2, (a) 1977, (b) 1978.

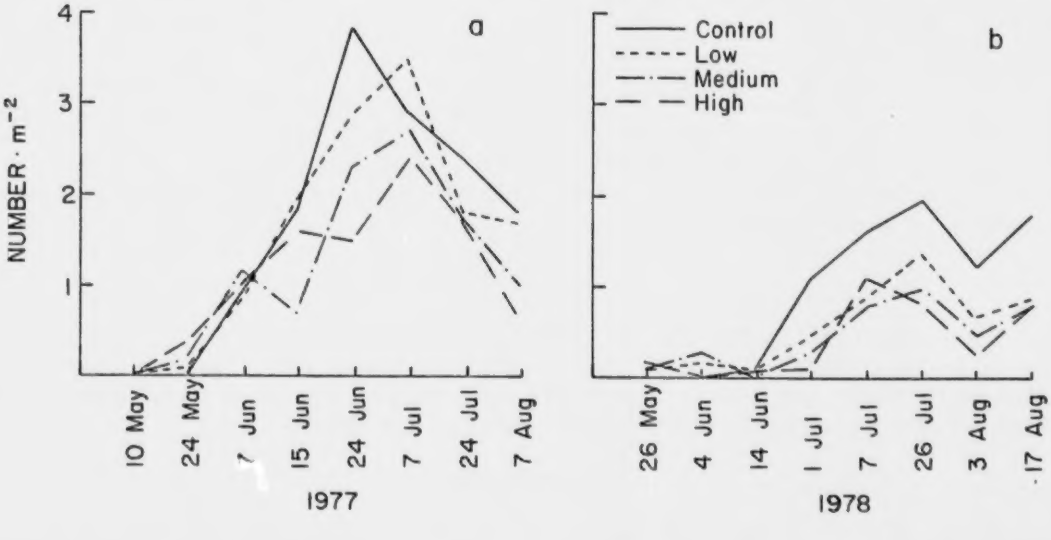

Figure 20.2. Seasonal dynamics of *Melanoplus sanguinipes* (Fab.) population fumigated with four different levels of SO_2, (a) 1977, (b) 1978.

beetles avoided immigrating into the SO_2 treatments at the ZAPS sites (Bromenshenk and Gordon, 1978). A relative increase in emigration of the grasshoppers may result from direct olfactory avoidance or from SO_2 making the forage impalatable.

Seasonal displacement rates for female *A. elliotti* averaged *ca.* 20 m (Mussgnug, 1972). Edwards (1961) concluded that *M. sanguinipes* moved only very short distances from a release point. Baldwin *et al.* (1958) found that the most actively tagged *M. sanguinipes* did not migrate outside a 10 m radius and none migrated more than 30 m from the point of release. Thus the treatment areas are larger than the normal displacement range of the grasshoppers. Therefore, the reduction in the populations could be attributed to direct contact with the pollutant or from indirect effects of the pollutant on other components of the ecosystem. Weedon *et al.* (1939) and Kanaga (1956) have established the direct toxicity of high SO_2 concentrations to insects. The concentrations used were higher than the ZAPS studies. No research has been done on direct toxicity from a long-term (chronic) pollution source.

The SO_2 could affect the grasshopper population indirectly by its effects on the grasshopper's predators and parasites in other insect populations. Hillman and Benton (1972) suggest that SO_2 disrupts the host-parasite balance resulting in decreased parasitic wasps and increased aphids. Freitag *et al.* (1973b) showed a reduction in predatory carabid beetles near a Kraft mill. This may cause a rapid increase in prey populations. But it has been suggested that grasshopper populations are not limited by predators and parasites (White, 1976). Therefore, it is possible that the population decrease resulted from the effects of SO_2 on the forage of the grasshoppers. Decreases in food quality and quantity consumed has been shown to decrease egg production in *M. sanguinipes* (F.) (Pfadt and Smith, 1972).

Since many chemicals have been shown to control feeding behavior and survival (Harley and Thorsteinson, 1967), the changes in forage chemistry because of air pollution could result in insect population changes. The total sulfur in western wheatgrass was four times greater on the high treatment than on the control at the end of the growing season. There was also a reduction in crude protein in the treated plots at Site I in 1976 (Schwartz *et al.*, 1978) and a reduction in the chlorophyll content (Lauenroth and Dodd, in prep.). These and other changes in the forage chemistry may be responsible for the decreases in grasshopper population.

CONCLUSIONS

The fumigation by SO_2 on the ZAPS sites caused a decrease in the population of grasshoppers. The non-statistically significant trend was lower population with higher concentration of SO_2 and length of fumigation. The specific action of SO_2 reducing the population was not determined.

REFERENCES

Baldwin, W. F., F. D. Riordan, and R. W. Smith. 1958. Note on Dispersal of Radioactive Grasshoppers. Can. Entomol., 90:374-376.

Bishop, J. A., L. M. Cook, J. Muggleton, and M. R. D. Seaward. 1975. Moths, Lichens and Air Pollution Along a Transect from Manchester to North Wales. J. Appl. Ecol., 12: 83-93.

Bromenshenk, J. J., and C. C. Gordon. 1978. Terrestrial Insects Sense Air Pollutants. In: Conference Proceeding, Forest Joint Conference on the Sensing of Environmental Pollutants, 1977. Am. Chem. Soc., Wash., D. C. pp. 66-70.

Brusven, M. A. 1967. Differentiation, Ecology and Distribution of Immature Slant-face Grasshoppers (Acridinae) in Kansas. Tech. Bull. 149. Agr. Exp. Sta. Kansas State Univ., 59 pp.

Brusven, M. A. 1972. Differentiation and Ecology of Common Catantopinae and Crytacanthacridinae Nymphs (Orthoptera: Acrididae) of Idado and Adjacent Areas. Melanderia, 9:32 pp.

Creed, E. R. 1975. Melanism in the Two Spot Ladybird: The Nature and Intensity of Selection. Proc. R. Soc. Lond. Ser. B., 190:135-148.

Edwards, R. L. 1961. Limited Movement of Individuals in a Population of the Migratory Grasshopper *Melanoplus bilituratus* (Walker) (Acrididae) at Kamloops, British Columbia. Can. Entomol., 93:628-631.

Freitag, R., and L. Hastings. 1973a. Kraft Mill Fallout and Ground Beetle Populations. Atmos. Environ., 7:587-588.

Freitag, R., L. Hastings, W. R. Mercer, and A. Smith. 1973b. Ground Beetle Populations near a Kraft Mill. Can. Entomol., 105:299-310.

Harley, K. L. S., and J. J. Thorsteinson. 1967. The Influence of Plant Chemicals on the Feeding Behavior, Development, and Survival of the Two Striped Grasshopper, *Melanoplus bivittatus* (Say) (Acrididae: Orthoptera). Can. J. Zool., 45:305-318.

Hillman, R. C., and A. W. Benton. 1972. Biological Effects of Air Pollution on Insects, Emphasizing the Reactions of the Honey Bee (*Apis mellifera* L.) to Sulfur Dioxide. Elisha Mitchell. Sci. Soc., 88:195.

Kanaga, E. E. 1956. An Evaluation of the Use of Sulfur Dioxide in Fumigant Mixtures for Grain Treatment. J. Econ. Entomol., 49:723-729.

Kettlewell, H. B. D. 1955. Selection Experiments on Industrial Melanism in the Lepidoptera. Heredity, 9:323-342.

Kettlewell, H. B. D. 1956. A Resume of Investigations of the Evaluation of Melanism in the Lepidoptera. Proc. R. Soc. Lond. Ser. B., 145:297-303.

Kettlewell, H. B. D. 1961. The Phenomenon of Industrial Melanism in Lepidoptera. Ann. Rev. Entomol., 6:245-262.

Lauenroth, W. K., and J. L. Dodd. Chlorophyll Reduction in Western Wheatgrass Exposed to Sulfur Dioxide. (In prep.).

Lees, D. R., E. R. Creed, and J. R. Duckett. 1973. Atmospheric Pollution and Industrial Melanism. Heredity, 30:227-232.

Mussgnug, G. L. 1972. The Structure and Performance of an Adult Population of *Aulocara elliotti* (Thomas) (Orthoptera, Acrididae) near Billings, Montana. M. S. Thesis, Montana St. Univ., 97 pp.

Pfadt, R. E. 1977. Some Aspects of the Ecology of Grasshopper Populations Inhabiting the Shortgrass Plains. In: Kulman, H. M., and H. C. Chiang, eds. 1977. Insect Ecology--papers presented in the A. C. Hodson Lectures. U. Mn. Agr. Expt. Sta. Tech. Bul. 310, 107 pp.

Pfadt, R. E., and D. S. Smith. 1972. Net Reproductive Rate and Capacity for Increase of the Migratory Grasshopper, *Malanoplus sanguinipes* (F.). Acrida, 1:149-165.

Schwartz, C. C., W. K. Lauenroth, R. K. Heitschmidt, and J. L. Dodd. 1978. Effect of Controlled Levels of Sulfur Dioxide in the Nutrient Quality of Western Wheatgrass. J. Appl. Ecol., 15:869-874.

Scoggan, A. C., and M. A. Brusven. 1972. Differentiation and Ecology of Common Immature Gomphocerinae and Oedipodinae (Orthoptera: Acrididae) of Idaho and Adjacent Areas. Melanderia, 8:77 pp.

Weedon, F. R., A. Hartzell, and C. Setterstrom. 1939. Effects on Animals of Prolonged Exposure to Sulfur Dioxide. Contrib. Boyce Thompson Inst., 10:281-324.

White, T. C. R. 1976. Weather, Food and Plagues of Locusts. Oecologia, 22:129-134.

SECTION 21

THE EFFECTS OF LOW CONCENTRATIONS OF SULFUR DIOXIDE ON THE GROWTH, QUALITY AND NITROGEN FIXATION OF ALFALFA

D. T. Tingey, G. E. Neely and M. L. Gumpertz

ABSTRACT

The effects of ambient concentrations of sulfur dioxide on the growth, yield, quality and nitrogen fixation of alfalfa *(Medicago sativa* L.*)* were determined. Plants were exposed throughout the growing season to median sulfur dioxide concentrations of 0, 2, 6, 15 or 20 pphm. Sulfur dioxide at 15 and 20 pphm significantly reduced plant growth, yield and nitrogen fixation. Total sulfur in the forage increased proportionally to the median sulfur dioxide concentration. No signficant effects in forage quality were observed.

INTRODUCTION

Increased sulfur dioxide emissions from power generation and other industrial processes pose an increased threat to the production of forage. Bell and Clough (1973) reported that continuous exposures of 6.7 and 12 pphm sulfur dioxide for 26 and 9 weeks, respectively, decreased the yield of ryegrass approximately 50%. *Dactylis* (Ashenden, 1978) exposed to 11 pphm sulfur dioxide for 4 weeks exhibited significant reductions in the number of tillers, leaf area and dry weights of leaves and roots. Katz and Ledingham (1939), summarizing a series of experiments, concluded that chronic exposures of 10 to 30 pphm sulfur dioxide for extended periods would have no effect on the yield of alfalfa under normal conditions. Intermittent exposures to 10 pphm sulfur dioxide for 7 hr per day 6 days per week through the growing season had no effect on alfalfa forage yield, but there was a tendency toward reduced root weight (Thomas *et al.*, 1943). More recently, however, Tingey and Reinert (1975) reported significant reductions in alfalfa forage and root growth by exposure to 5 pphm SO_2 (8 hr/day, 5 days/week) from seeding to harvest. Sulfur dioxide-induced reduction in root dry weight in grass (Ashenden, 1978) and alfalfa (Thomas *et al.*, 1943; Tingey and Reinert, 1975) indicates that the roots received less photosynthate. This suggests that sulfur dioxide exposures could affect processes that require an input of photosynthate, such as symbiotic nitrogen fixation.

Howell (1975) exposed alfalfa to 150 pphm sulfur dioxide for 2 hr and analyzed the forage 36 hr later for indicators of foliage quality. He reported significant reductions in the protein and fat content and increases in the total carbohydrate and fiber content. However, at lower concentrations (10 to 40 pphm for 1 to 14 days) there were no effects on either the nitrogen fraction or the carbohydrates in alfalfa (Katz and Pasternak, 1939).

The objectives of this study were (1) to assess the effects of continuously varying sulfur dioxide concentrations, which simulate ambient exposures, on the growth and yield of alfalfa; (2) to determine the effects of sulfur dioxide on indicators of forage quality; and (3) determine the impact of sulfur dioxide on symbiotic nitrogen fixation.

MATERIALS AND METHODS

Plant Growth and Exposure.

Alfalfa (*Medicago sativa* L.) cv Mesa-Sirsa seeds were inoculated with a commercial *Rhizobium* inoculum and planted in 20 mesh silica sand in 22 cm dia. plastic pots and watered daily with a modified Hoagland nutrient solution, minus nitrogen. To promote rapid plant growth, the nutrient solution was supplemented with 220 ppm N during the first two weeks and during the third week with 110 ppm N. After emergence, the plants were thinned to two individuals per pot.

Plants were placed in field exposure chambers (10 pots/chamber) (Heagle *et al.*, 1973) when approximately 10 cm tall and exposed to a simulated sulfur dioxide time series for 68 days. A gradient in median sulfur dioxide concentration was designed with a control and four concentrations of sulfur dioxide (Table 21.1). The exposure simulation duplicated the correlation structure of ambient air monitoring data (Air Quality Criteria for Sulfur Oxides, 1970) and had a standard geometric deviation of 2.44 (Male *et al.*, 1978; Male, 1980). The sulfur dioxide concentrations used in the time series exposure were generated using a lognormal stochastic model. The same exposure pattern (sulfur dioxide concentrations increased and decreased at the same time in all the treatment chambers) was used in all treatment chambers except the median sulfur dioxide concentrations were different. The sulfur dioxide levels were varied daily at hourly intervals between 8:00 a.m. and 4:00 p.m. PDT. During the remaining 16 hr period the sulfur dioxide concentration remained constant at the median concentration calculated from the stochastic model for that 16 hr period. The median sulfur dioxide concentrations for each harvest are shown in Table 21.1. Sulfur dioxide was dispensed and controlled in the field exposure chambers as described by Heagle *et al.* 1974. It was monitored using flame photometric detectors which were calibrated daily using sulfur dioxide permeation tubes.

The plants were exposed for 35 and 33 days, respectively, prior to the first and second harvests. All plants were harvested when the plants in the control chamber reached the 1/10 bloom growth stage. Tops were clipped 8 cm above the growth medium; this is subsequently referred to as the top or forage portion. At the second harvest, the forage was once again clipped at the 8 cm level and the remaining plant material was separated into stubble and roots. After harvest the plant parts were dried at 70°C until the material reached a constant weight.

TABLE 21.1. MEDIAN SULFUR DIOXIDE CONCENTRATIONS (PPHM).

Desired (nominal)	Actual Median 1st Harvest	Actual Median 2nd Harvest	Actual Median Seasonal
0	0.0	0.0	0.0
2	2.1	2.4	2.3
6	6.0	5.6	5.8
15	17.2	17.9	17.6
20	13.9	22.8	18.6

Chemical Analysis.

The dried plant material was ground in a Wiley Mill (40 mesh screen); aliquots were used for the various metabolite analyses. To determine Kjeldahl nitrogen, samples were digested (Taras *et al.*, 1971) and the resulting ammonia was measured using the phenol-hypochlorite reaction (U.S. Environmental Protection Agency, 1974). Total nonstructural carbohydrates were extracted and analyzed using the method of Smith (1969). Total sulfur was estimated by digesting the plant material in 70% nitric and perchloric acid; the resulting sulfate was determined turbidimetrically (Rand *et al.*, 1976). Dry matter digestibility of the forage was estimated using the *in vitro* method of Tilley and Terry (1963).

Data Analysis.

Preliminary data analyses indicated that the variances of the response variables increased with the means for all responses except digestibility. Therefore, the data were transformed to their respective logarithms to stabilize the variances. All statistical analyses except those for digestibility were performed on the transformed data. The anti-logarithms of the data were computed to simplify the presentation of results. Data from each harvest were subjected to individual statistical analysis because the median sulfur dioxide concentrations for the two harvests differed (Table 21.1).

The data from the first harvest were subjected to analyses of variance to determine if there was an effect of sulfur dioxide on any of the response variables. Data from the second harvest were subjected to analyses of variance to determine if the sulfur dioxide treatment affected the response variables or the plant parts differently. When an analysis of variance yielded a significant F-test, at $\alpha = 0.05$, the Bonferroni method of multiple comparisons was used to determine which treatment means differed significantly from the control mean. For all response variables except digestibility, the test for determining if a particular treatment mean was significantly different from the control mean consisted of comparing the ratio $x_{control}/x_{treatment}$ with a critical value. For digestibility, the differences $| \bar{x}_{control} - \bar{x}_{treatment} |$ were compared with a critical value. The critical values from the Bonferroni multiple comparison procedures are shown in the appropriate figure legends.

RESULTS

Forage dry weight of the control plants for harvest 2 was approximately twice that of harvest 1 (Figure 21.1A) indicating better plant growth. For both harvests, forage dry weight for the lowest two sulfur dioxide concentrations was similar to or slightly larger (not significant) than the controls. However, higher concentrations of sulfur dioxide elicited significant reductions in forage dry weight (yield). Foliar injury was observed in both harvests at the higher sulfur dioxide concentrations (15 and 20 pphm, nominal). Visually, the plants in the 6 pphm treatment appeared to have fewer leaves and more spindly stems. However, these visual symptoms were not reflected in a significant effect on plant yield.

At harvest 2 the sulfur dioxide treatment impacts were measured on the forage, stubble, roots and total plant dry weight (Figure 21.1B). There were no significant decreases in the growth of any plant part at the 2 or 6 pphm sulfur dioxide treatment but significant growth reductions for all plant parts occurred at the higher sulfur dioxide concentrations.

In harvest 2, the proportion of dry weight in the root, stubble and forage was determined. Across treatments, the proportion of the plant dry weight in the roots was essentially constant (approximately 42%). The proportion of the dry weight in the stubble increased from approximately 18% for the control plants to approximately 30% at the highest sulfur dioxide treatment. The proportion of dry weight in the forage decreased from approximately 39% in the control plants to approximately 25% at the highest sulfur dioxide treatment. This decrease in dry matter partitioning into the forage may, in part, account for the visual observation of weaker stems and less leaf matter in the 6 pphm treatment.

The concentration of nitrogen, total non-structural carbohydrates, total sulfur and *in vitro* dry matter digestibility were determined to estimate the impact of the sulfur dioxide on forage quality (Figure 21.2). In the control plants, for both harvests 1 and 2, the nitrogen content was 3.2 and 3.4%, respectively (Figure 21.2A) which is equivalent to protein contents of approximately 20 and 21%. There was no significant change in the nitrogen content of the forage with respect to sulfur dioxide concentration for harvest 1. However, for harvest 2 there was an approximately linear increase in nitrogen content with sulfur dioxide concentration.

Total nonstructural carbohydrates (Figure 21.2B) tended to be higher for harvest 2 than for harvest 1. Although no significant differences in carbohydrate content were detected between treatments, the carbohydrate contents tended to increase with increasing sulfur dioxide concentrations in harvest 1 and were variable in harvest 2 with a tendency to decrease with increasing sulfur dioxide concentration.

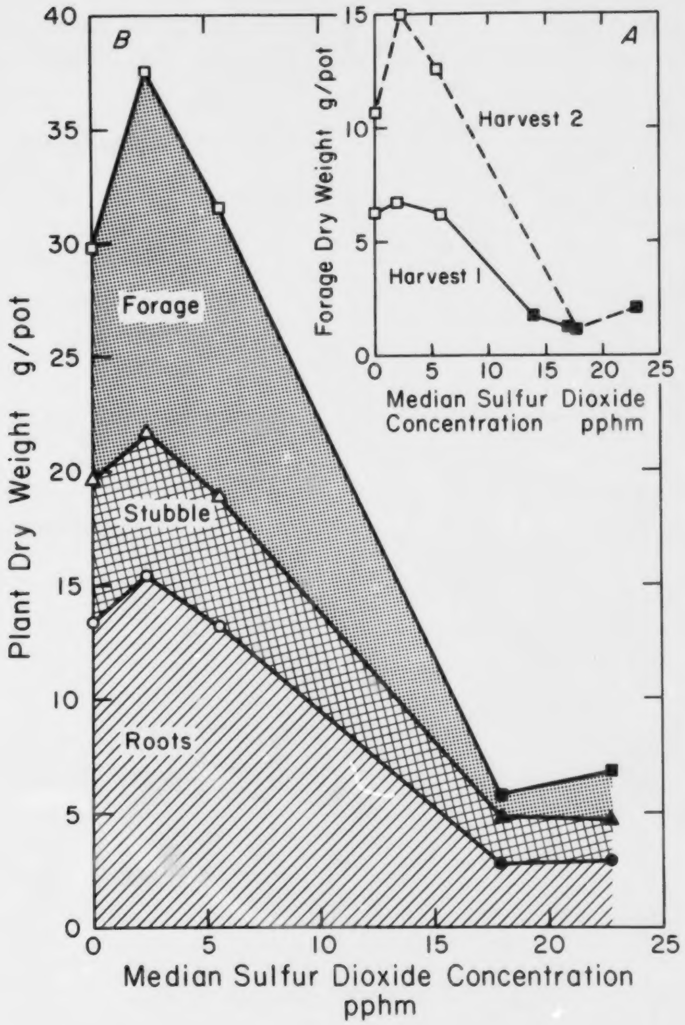

Figure 21.1. The influence of sulfur dioxide on the growth and yield of alfalfa. Each mean is based on 10 observations. Open symbols indicate that the treatment mean was not significantly different from the control ($\alpha = 0.05$). (A) The yield of alfafa for each harvest. The critical values are 1.66 and 1.62 for harvest 1 and 2, respectively. (B) The growth of the individual plant components and total plant growth for harvest 2. The top line describes the summed weights of the individual plant components and is equal to total plant growth. The weights of the individual plant parts are indicated by the appropriate cross-hatched areas. The critical value for the individual components is 1.62 and total growth is 1.42.

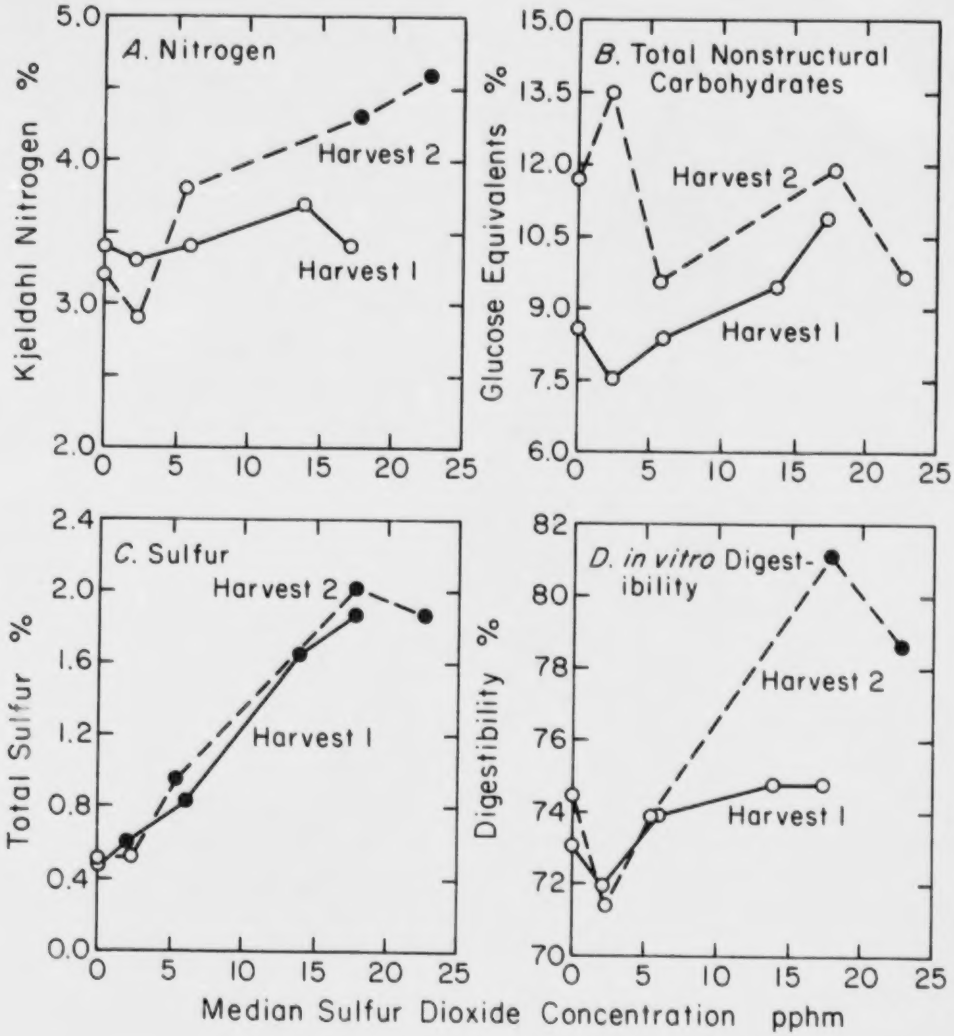

Figure 21.2. The influence of sulfur dioxide on indicators of forage quality. Each mean is based on 10 observations. Open symbols indicate that the treatment mean was not significantly different than the control while solid symbols indicate that the mean was significantly different from the control ($\alpha = 0.05$). (A) Nitrogen content of the forage. The critical values for harvests 1 and 2 are 1.12 and 1.31. (B) Forage content of total nonstructural carbohydrates. The critical values for harvest 1 and 2 are 1.29 and 1.37. (C) The total sulfur content of the forage. The critical values for harvests 1 and 2 are 1.19 and 1.40. (D) The disappearance of dry matter or digestibility. For harvest 1, the analysis of variance indicated no significant differences. For harvest 2 the critical value is 3.41.

Total sulfur content in the forage increased in an approximately linear manner with increasing sulfur dioxide concentration (Figure 21.2C) for both harvests. The sulfur content ranged from approximately 0.46 and 0.47% for the controls to a high of approximately 1.8% for the highest sulfur dioxide concentration for each harvest. The concentration of sulfur in the stubble showed a similar trend to that of the foliage but the content was approximately half that in the forage. The sulfur concentration in the control plant roots was approximately 0.69%, but when the sulfur dioxide concentration exceeded approximately 6 pphm, the concentration in the roots decreased to approximately 0.45%.

In vitro dry matter digestibility (Figure 21.2D) for harvest 1 was not significantly different among treatments and averaged 73.7%. The digestibility for harvest 2 was similar to harvest 1 for the control and the lowest two sulfur dioxide treatments, however, there were significant increases in digestibility to 81.4 and 78.7%, respectively, for the highest two sulfur dioxide treatments.

To estimate symbiotic nitrogen fixation, plants were inoculated with a *Rhizobium* culture and grown in a nitrogen-free medium. Using this method the nitrogen content of the plant depends on nitrogen derived from the atmosphere via symbiotic nitrogen fixation. The total nitrogen fixed was estimated by determining the nitrogen content of the plant tissue and the total amount of plant tissue produced. The control plants fixed approximately 1050 mg/pot of nitrogen for the season (Figure 21.3). There was a nonsignificant increase in nitrogen fixation at the 2 and 6 pphm levels. In the 15 and 20 pphm treatments large decreases in nitrogen fixation occurred.

The total nonstructural carbohydrate content of the stubble and roots were analyzed to determine if sulfur dioxide altered the concentration (Figure 21.4). Total nonstructural carbohydrates in the forage tended to increase slightly in harvest 1 and decrease in harvest 2 although the results for both harvests were somewhat variable (Figure 21.2B) and the differences were not significant. In contrast, the concentration of total nonstructural carbohydrates in the stubble was similar to the control except at the highest sulfur dioxide concentration, which was significantly less. The nonstructural carbohydrate content of the roots was similar to or slightly less than the control at the lowest two sulfur dioxide treatments. Higher concentrations of sulfur dioxide elicited significant reductions in the carbohydrate content in the roots. These results indicate that sulfur dioxide significantly reduced the partitioning of carbohydrate into the roots.

DISCUSSION

Plant growth and yield in the low concentration sulfur dioxide treatments tended to be greater than the control but not significant statistically. Thomas *et al.* (1943) also reported a nonsignificant stimulation of alfalfa growth from low concentrations of sulfur dioxide. The trend toward increased growth observed in our study was not the result of the atmospheric sulfur dioxide overcoming a sulfur deficiency. Foliar analysis (Figure 21.2C) indicated that sulfur content exceeded 0.45% which is much greater than 0.20 to 0.22% established as the critical level for adequate sulfur nutrition (Bickoff

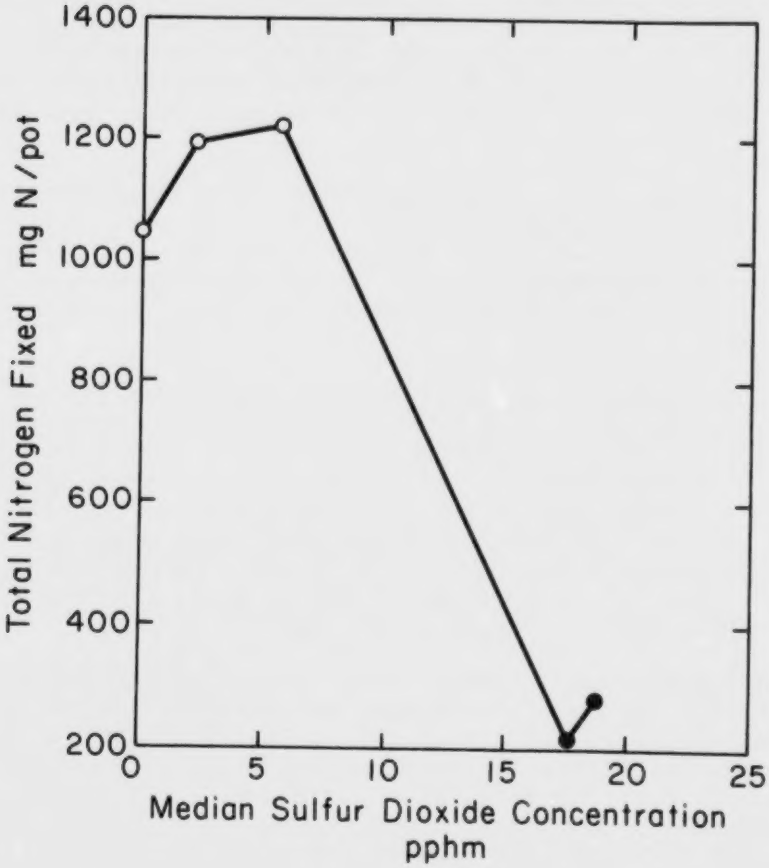

Figure 21.3. The effect of sulfur dioxide on total nitrogen fixation by alfalfa plants for the growing season. Open symbols indicate that the treatment mean was not significantly different than the control while solid symbols indicate that the mean was significantly different from the control ($\alpha = 0.05$). Each mean is based on 10 observations and critical value is 1.49.

et al., 1972). Low concentrations of sulfite, an oxidation product of sulfur dioxide in plant tissue, will stimulate photosynthesis (Libera *et al.*, 1973); this stimulation may explain the trend toward increased growth observed in the low concentrations of sulfur dioxide.

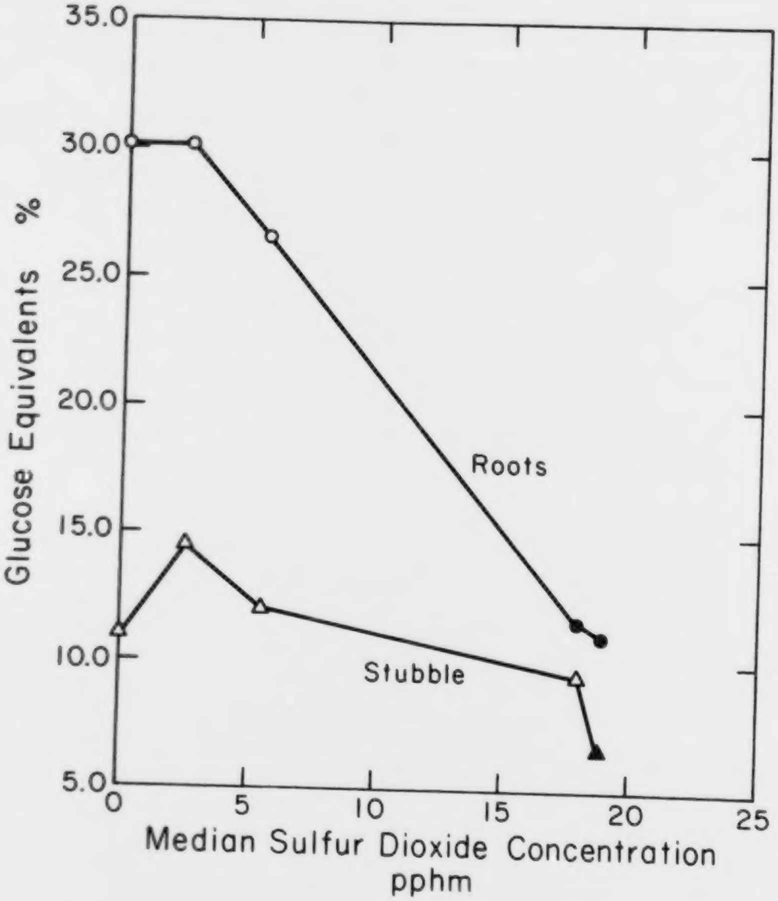

Figure 21.4. The influence of sulfur dioxide on the total nonstructural carbohydrate content of the stubble and roots. Open symbols indicate that the treatment mean was not significantly different from the control while solid symbols indicate that the mean was significantly different from the control ($\alpha = 0.05$). Each mean is based on 10 observations and the critical value is 1.37. Data were collected at harvest 2.

Plant growth was reduced at lower sulfur dioxide concentrations in our study than has been reported for other chronic field studies with alfalfa (Thomas *et al.*, 1943; Katz and Ledingham, 1939). A more sensitive cultivar may have been used in our study; or the continuous exposures to fluctuating sulfur dioxide concentrations, rather than a constant level as in previous studies may have caused effects at lower concentrations.

The reduced partitioning of carbohydrates into the roots is similar to previous reports for cocksfoot (Ashenden, 1978) and alfalfa (Thomas *et al.*, 1943). Short term exposures to high sulfur dioxide concentrations have been reported to reduce both photosynthesis and the translocation of photosynthate from the leaves to other sinks (Teh and Swanson, 1979). A reduction in photosynthate translocation could account, in part, for the reduced levels of photosynthate in the roots of the alfalfa plants. The roots accumulate carbohydrate only in excess of that needed to maintain foliar growth (Brown *et al.*, 1972; Smith and Marten, 1970). The reduction in storage carbohydrates in the roots may have long-range significance for the vigor and the production of the crop. Root carbohydrate reserves provide the energy necessary for foliar growth of alfalfa following defoliation (harvest).

The forage quality parameters of the control plants were similar to those reported for field grown alfalfa crops (Bickoff *et al.*, 1972). In our study, sulfur dioxide had little effect or caused a slight increase in protein content, and caused no distinct change in the carbohydrate content. The increased nitrogen content in harvest 2 is of little significance because the forage production was significantly less so the total amount of protein available per unit of ground area was also reduced significantly.

Katz and Pasternak (1939) reported no change in the nitrogen fractions or carbohydrates of alfalfa exposed to 10 to 40 pphm sulfur dioxide for 1 to 14 days. Howell (1975) reported that short term exposures to high concentrations of sulfur dioxide reduced the protein content and increased the carbohydrate content of forage. The digestibility of all our samples was high and the slight but significant increase with the high sulfur dioxide treatments would have no practical value. The lack of significant effects on forage quality in our study is similar to results reported by Cunningham *et al.* (1937); they reported no difference in performance of dairy cows fed either control alfalfa or alfalfa with less than 25% of the leaf area showing sulfur dioxide injury.

The total sulfur content of the alfalfa foliage increased proportionally to the sulfur dioxide concentration. Faller *et al.*, (1970) reported that inorganic sulfur was the major sulfur component accumulated in plant foliage from sulfur dioxide fumigations. Inorganic sulfur presumably accounted for the large total sulfur increase observed in our alfalfa forage. The majority of the sulfur dioxide absorbed from the atmosphere was retained in the forage and lesser amounts were exported to stems and roots (Faller *et al.*, 1970; Yamazoe, 1973). Also, plants fumigated with sulfur dioxide absorbed less sulfur through the roots (Thomas *et al.*, 1943, Scharer *et al.*, 1975). The suppression of sulfur uptake from the soil and the low export of sulfur from the leaves to the roots could explain the decreased sulfur content of the roots with increased sulfur dioxide exposure observed in our study.

Our results indicate that symbiotic nitrogen fixation was suppressed at the higher sulfur dioxide concentrations. The reduction in nitrogen fixation is expected since metabolites from leaves and stems are translocated to the roots and the carbohydrate levels of the roots were reduced. Carbohydrates provide the energy needed to reduce and incorporate atmospheric nitrogen into organic forms (Gibson, 1976). This suggests that the reduction in carbohydrate translocated to the alfalfa roots decreased the amount of nitrogen fixed and thus reduced, in part, plant growth and yield.

CONCLUSIONS

Sulfur dioxide suppressed alfalfa growth, yield and nitrogen fixation only at the highest two concentrations (15 and 20 pphm). Total sulfur in the forage increased proportionally to the median sulfur dioxide concentration. Forage quality was not impaired by the sulfur dioxide exposures.

REFERENCES

Air Quality Criteria for Sulfur Oxides. 1970. National Air Pollution Control Administration. Washington, DC. AP-50.

Ashenden, T. W. 1978. Growth Reductions in Cocksfoot (*Dactylis glomerata* L.) as a Result of SO_2 Pollution. Environ. Pollut. 15:161-166.

Bell, J. N. B. and W. S. Clough. 1973. Depression of Yield in Ryegrass to Sulfur Dioxide. Nature, 241:47-49.

Bickoff, E. M., G. O. Kohler and D. Smith. 1972. Chemical Composition of Herbage. In: C. H. Hanson, ed. Alfalfa Science and Technology. American Society of Agronomy, Inc. Madison, Wisconsin. pp. 247-282.

Brown, R. H., R. B. Pearce, D. D. Wolf and R. E. Blaser. 1972. Energy Accumulation and Utilization. In: C. H. Hanson, ed. Alfalfa Science and Technology. American Society of Agronomy, Inc. Madison, Wisconsin. pp. 143-166.

Cunningham, C. C., L. H. Addington and L. T. Elliot. 1937. Nutritive Value for Dairy Cows of Alfalfa Hay Injured by Sulfur Dioxide. J. Agri. Res., 55:381-391.

Faller, N., K. Herwig and H. Kuehn. 1970. The Absorption of Sulfur Dioxide ($^{35}SO_2$) From the Air. II. Absorption, metabolism and distribution in plants. Plant and Soil, 33:283-295.

Gibson, A. H. 1976. Limitation to Dinitrogen Fixation by Legumes. In: Nitrogen Fixation. W. E. Newton and C. J. Nyman, eds. Washington State University Press, Pullman, pp. 400-428.

Heagle, A. S., D. E. Body and W. W. Heck. 1973. An Open-Top Field Chamber to Assess the Impact of Air Pollution on Plants. J. Environ. Quality, 2:365-368.

Heagle, A. S., D. E. Body and G. E. Neely. 1974. Injury and Yield Responses of Soybean to Chronic Doses of Ozone and Sulfur Dioxide in the Field. Phytopathology, 64:132-13.

Howell, R. K. 1975. Ozone and Sulfur Dioxide--Their Effect on Nutrient Composition of Forages. Reg. Conf. Rumen Funct. 1975:4-5.

Katz, M. and G. A. Ledingham. 1939. Experiments on Yield of Barley and Alfalfa. Chapter XIII. Effect of Sulfur Dioxide on Vegetation. National Research Council of Canada. pp. 322-368.

Katz, M. and D. S. Pasternak. 1939. Effect of Fumigation on Some Chemical Constituents of Barley, Wheat and Alfalfa. Chapter XIV. Effect of Sulfur Dioxide on Vegetation. National Research Council of Canada. pp. 369-392.

Libera, W., H. Ziegler and I. Ziegler. 1973. Förderung der Hill-Reaktion und der CO_2-Fixierung in Isolierten Spinatchloroplasten durch Niedere Sulfitkonzentrationen. Planta, 109:269-279.

Male, L. 1980. Time Series Experiments for Predicting Plant Growth Response to Pollution. Part I.--Time Series Model and Experimental Design. Atmospheric Environment (Submitted).

Male, L., J. Van Sickle and R. Wilhour. 1978. Time Series Experiments for Studying Plant Growth Response to Pollution. EPA-600/3-78-038. U.S. Environmental Protection Agency, Corvallis, OR.

Rand, M. C., A. E. Greenberg and M. J. Taras. 1976. 427 C. Turbidimetric Method. In: Standard Methods for the Examination of Water and Wastewater, 14th edition. American Public Health Association, Washington, DC. pp. 496-498.

Scharer, M., C. Brunold, and K. H. Erismann. 1975. Hemmung der Sulfataufnahme durch *Lemna minor* L. durch SO_2 in Subletalen Konzentrationen, Experientia, 31:1414-1415.

Smith, D. 1969. Removing and Analyzing Total Nonstructural Carbohydrates from Plant Tissue. Research Report 41. University of Wisconsin, Madison Wisconsin.

Smith, L. H. and G. C. Marten. 1970. Foliar Regrowth of Alfalfa Utilizing ^{14}C-labeled Carbohydrates Stored in Roots. Crop Science, 10:146-150.

Taras, M. J., A. E. Greengerg, R. D. Hoak and M. C. Rand, eds. 1971. Nitrogen (organic). In: Standard Methods of the Examination of Water and Wastewater, 13th edition. American Public Health Association, Washington, DC. pp. 244-248

Teh, K. H. and C. A. Swanson. 1979. Comparative Inhibition of Photosynthesis and Translocation by Sulfur Dioxide in Bush Bean. Plant Physiology (supplement), 63:34.

Thomas, M. D., R. E. Hendricks, T. R. Collier and G. R. Hill. 1943. The Utilization of Sulphate and Sulfur Dioxide for the Sulfur Nutrition of Alfalfa. Plant Physiol., 18:345-371.

Tilley, J. M. A. and R. A. Terry. 1963. A Two Stage Technique for the *in vitro* Digestion of Forage Crops. J. British Grassl. Soc., 18:104-111.

Tingey, D. T. and R. A. Reinert. 1975. The Effect of Ozone and Sulfur Dioxide Singly and in Combination on Plant Growth. Environ. Pollut., 9:117-125.

U.S. Environmental Protection Agency. 1974. Methods for Chemical Analysis of Water and Wastes, Washington DC. 298 pp.

Yamazoe, F. 1973. Distribution and Reaction of Sulfur Dioxide after Absorption by Plants. Jap. Agric. Res. Q., 7:243-247.

SECTION 22

REMOTE SENSING OF SO_2 EFFECTS ON ZAPS

J.E. Taylor, W.C. Leininger, and M.W. Hoard

ABSTRACT

Color infrared aerial photographs were taken during June, August and October 1978 on the ZAPS sites. 1:15,000 photos were taken as synoptic records of the third and fourth years of sulfur dioxide fumigation effects of ZAPS II and I, respectively. Larger scale photography (1:3,700) was analyzed visually and densitometrically for color and density differences associated with SO_2. Visual bleaching with increasing fumigation rates was observed on both sites at all dates; this effect was most conspicuous in the August sample. Densitometric data indicated a general tendency for decreased color intensity in red, green, and blue wave lengths. Total density likewise decreased across treatments, but red/green ratios were insensitive. Although intermediate levels varied, significant density reductions were seen between control and high SO_2 rates at every sample date. Significant linear regressions related stress and response in several comparisons, especially in the red wave lengths. This indicates a loss of actively growing plant material. Ground-level photographs were taken on the ZAPS photo plots and on the fertilizer study conducted by Colorado State University. Aspect views for visual comparisons among treatments and years also were taken.

INTRODUCTION

We have been using remote sensing to monitor the effects of SO_2 on vegetation on the ZAPS and in the vicinity of Colstrip since 1974. Ground-level and aerial photography have been used in our monitoring efforts. Ground-level photo techniques and data for the Colstrip area are discussed in section 4 ; only data on the ZAPS are reported herein.

MATERIALS AND METHODS

Aerial Photography

Procedures

In our aerial photography we have concentrated on developing procedures which use standard, readily available equipment, since if a system is to be applied in a variety of situations it must not necessitate highly specialized, costly investments.

For our overflights we use a Cessna 182 airplane which can easily handle the required elevation range of 500 to 7,000 feet above the ground. The plane we use is leased from Miles City Aero Services, Miles City, Montana. This aircraft has been modified by the addition of a 30.5 cm diameter belly port, which accepts a special camera mount, designed and manufactured by W.E. Woodcock of Miles City (Woodcock, 1976). The camera is fitted with a 50 or 80 mm lens depending on the desired photo scale.

In addition to the mounted camera, a second camera is used for making oblique photographs from the air. This kind of photography supplements the more traditional vertical imagery, in that it is more representative of familiar aspects of scenes for interpretation, display, etc.

We use various photo scales, depending upon the amount of ground detail (resolution) and the area per frame (coverage) required for different purposes. All of our vertical aerial photography is flown with 60% end lap so it can be viewed stereoscopically.

Our largest scale is 1:3,700, which is obtained by flying at 305 meters above ground with an 80 mm lens. Larger scales are effectively precluded by the minimum 1/500 second shutter speed of the camera. At 195 km per hour, motion causes a slight displacement in the direction of flight. This is negligible at smaller scales, but becomes noticeable at lower elevations or longer lens focal lengths.

Even with image displacement, photography at this large scale shows excellent ground detail, discriminating individuals of many plant species and even 10 x 15 cm plastic marking flags and the wires supporting them.

When more coverage is desired and ground detail is less critical, flying altitude is increased or lenses changed to shorter focal lengths. For instance, to get each ZAPS location in one frame we use a scale of 1:15,000. This still allows good species discrimination and also reveals more of the surrounding areas for topographic analysis, drift detection, etc.

For optimum discrimination of infrared reflectance, CIR film requires a minus blue filter, which removes some of the shorter wave length radiation to which the film is also sensitive. The manufacturer's recommendation is a yellow (Wratten 12) filter. We prefer a Wratten 15, which is a little more orange colored, and which cuts off slightly more of the yellow-orange portion of the spectrum. In our situation, this improves the separation of infrared reflectances, which are rendered on CIR in tones of red. Also, it produces ground tones which are less blue than those obtained with the 12 filter, and we find these more visually pleasing.

In 1978, aerial photo missions were conducted over the ZAPS on June 28, August 8, and October 9. No aerial photography was taken of the Colstrip sites. Color I. R. film (Kodak #2443) was exposed at scales of 1:3,700 and 1:14,000 for all dates. In addition, color negative film (Kodak Vericolor VPS-120) was used at the 1:3,700 scale in June and both scales in October.

Film Processing

All CIR film is sent to Precision Photo[1/] in Dayton, Ohio for processing. An optical density step wedge is exposed onto the film end before processing for density and color calibration. The color negative film is commercially processed within the State.

Printing

We use the Cibachrome [1/] process for all infrared color printing because of its sharpness, good color saturation, and resistance to fading. We can produce these color prints in our laboratory. Prints from color negative film are made by us or by commercial firms.

Aerial Photo Analysis

Visual Observations

The simplest and easiest kind of photo interpretation (PI) of SO_2 effects is identification on the photographs of ground abnormalities. Shape, size, shadows, texture, and proximity to other objects all are clues for PI, but in color systems (including CIR), color is the most conspicuous and useful characteristic in detecting temporal and spatial changes. When we receive the photos from processing, a visual comparison is made between fumigation plots and adjacent vegetation to the plots. We examine color and density differences for plant communities and inspect the boundaries of the plots for possible drift patterns.

Densitometric Analysis

We also sample optical density within the film areas which represent the fumigation plots. This produces numerical data which can be used in regression and correlation expressions with other data types. It allows rigorous characterization of image colors.

The densitometer used in these analyses is a Macbeth TD 524 (Status A), with a 1 mm spot. This instrument reads the density of aerial transparencies in the red, blue, and green spectral ranges, and also gives a total density

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[1/] No endorsement of specific brand names is implied.

value for each observation. Transmission density, as analyzed here, refers to the proportion of light projected onto the transparency which passes through. Density is expressed as the common logarithm of opacity, which is defined as the reciprocal of transmittance. Thus, if 10 percent of the light striking the film is transmitted, opacity is 10 (1/0.1), and the density is 1.00 (log 10).

The density values which are yielded in this analysis are therefore related to the color saturation of the wave length being discussed. Thus, a high density for red indicates a high red content in the image.

In reading, 10 samples (of 1 mm diam.) are selected from each treatment area recorded on the film (5/north replication and 5/south replication). Red, green, blue and total density values are determined at each sample plot.

At the scale of 1:3,700, a 1 mm spot represents a ground area of 10.75 m^2. Thus, each reading integrates the reflectance exhibited by all parts of the scene. Gas delivery pipes, with their high reflectances, are avoided in placing the densitometer spot.

For the regression analyses, we calculated mean SO_2 rates from the start of the fumigation season until the time of photography (Table 22.1). We feel that since we are photographing current annual growth in the sites, the fumigation rates for current year should best represent the treatment levels influencing photographic reflectance.

TABLE 22.1. 1978 MEAN SO_2 RATES (PPHM) USED FOR REGRESSION ANALYSES*

Photography date	ZAPS I				ZAPS II			
	A	B	C	D	A	B	C	D
June 28	1.05	2.10	3.60	7.05	1.15	2.40	4.25	8.4
August 8	1.07	2.30	3.90	7.60	1.13	2.30	4.10	8.56
October 9	1.06	2.46	4.40	7.80	1.18	2.38	4.32	8.94

* Data from Dr. Eric Preston.

Ground-Level Photography

Permanently marked photo plots on the ZAPS were stereoscopically photographed with color film on June 18, August 3, and September 10, 1978. Details on photo plot monitoring procedures are given by Taylor *et al.*, 1976. Color aspect photos of the site from adjacent vantage points were also made. Detailed vegetation maps for the photo plots were constructed (see section 4 for details) with species identification on the photos to obtain quantitative data on species canopy coverage and frequency. These data are to be compared with similar data taken from the plot photographs and will be used to evaluate the feasibility of estimating these parameters from photo plots.

Photo plots were established and stereoscopically photographed (June 4 and July 16) on Colorado State University's fertilization study on ZAPS I. Plots were established on the southern replication only. Both color and color I. R. film was exposed in June, while only color I. R. film was taken in July. These photos were sampled densitometrically in order to provide digital data that can be used by C.S.U. in assessing the effects of SO_2 on grassland under varying regimes of sulfur and nitrogen.

RESULTS AND DISCUSSION

Aerial Photo Analysis

Visual Observations

Visual differences continue to be observed across fumigation plots at all seasons. With increasing SO_2 rates, treatment plots appear increasingly lighter in tone. This is most apparent at the peak of growth and is less noticeable as the vegetation matures. This is evident in the field as well as in both color and infrared color photography.

Densitometric Analysis

Photographic density values for the three 1978 aerial missions using CIR film are shown in Figures 22.1, 22.2 and 22.3 (June, August and October flights, respectively.)

The colors quantified by the densitometer are those produced in CIR film. The relationships among original and CIR image colors and some examples used in interpretations are given in Table 22.2.

June

ZAPS I shows a general decrease in density with fumigation for all colors. There was a slight rise in blue and green on the C (medium) plot, but the red content of the images, recording infrared reflectance, decreased linearly. Comparing the extreme rates, the high fumigation was significantly less dense than the control in most cases.

On ZAPS II, the decreased color densities with fumigation were more striking, especially between control and high rates.

August

The color content of the film was higher than in June, which could be due to different proportions of plant and substrate reflectance, or to differences in film exposure, and/or processing. Thus, comparisons of density

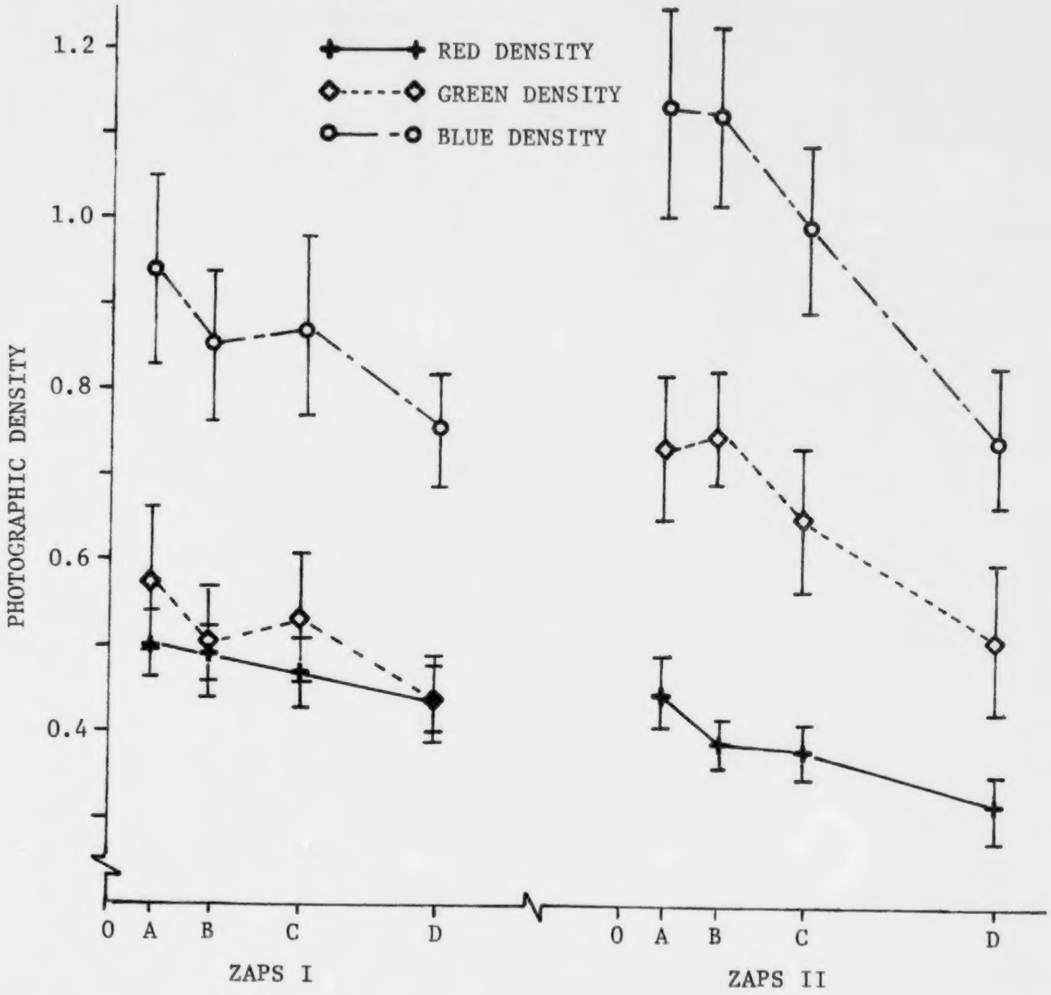


Figure 22.1. Densitometric readings on ZAPS plots; June 1978 (1:3,700 color infrared film)

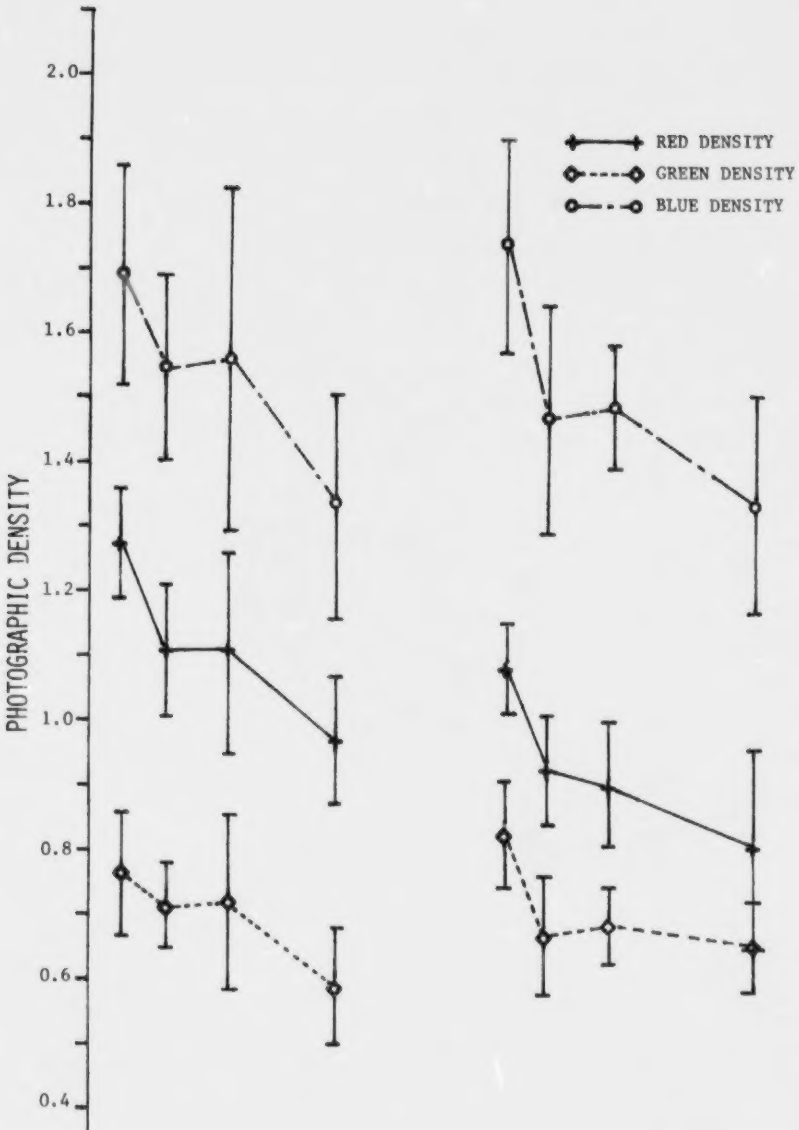


Figure 22.2. Densitometric readings on ZAPS plots; August 1978 (1:3,7000 CIR film)

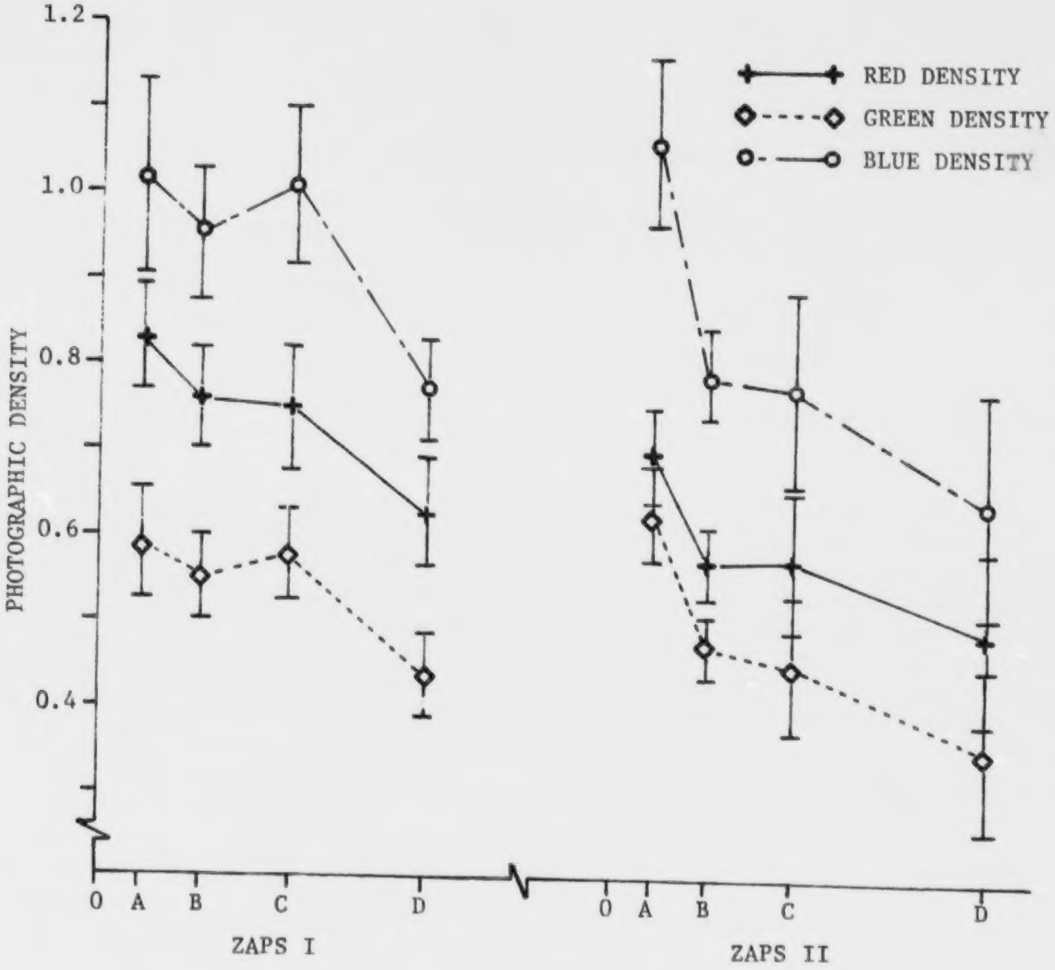


Figure 22.3. Densitometric readings on ZAPS plots; October 1978 (1:3,700 CIR film).

TABLE 22.2. RENDITION OF SUBJECT COLORS IN CIR (COLOR INFRARED) FILM

Original (natural) color		CIR rendition	
Wave lengths (nanometers)	Color rendition	Infrared rendition	Predominant wave length in densitometer
630-700	red	green	646
500-600	green	blue	545
400-500	blue	--	460

Examples of CIR records:

Healthy, vigorous vegetation	Red
Mature or diseased vegetation	Greenish
Litter	Green to yellow
Bare ground	White or green
Turbid water	Light blue
Clear water	Black

values among dates is not appropriate. When comparing within-date reflectances, trends observed in June were even more pronounced in August. Both a loss in color and a slight rebound at the C plot were again measurable across the fumigation rates. ZAPS II densities differed somewhat from the earlier date, particularly at the low (B) SO_2 level, where substantial color was lost relative to the control. Again, within-treatment variation was greater than at the June observation. This probably was caused by the more pronounced difference in maturity between warm and cool-season plant species at the latter time. In the field, the D (high) plot on ZAPS II looked even more chlorotic than the data suggest because of the highly bleached "hot spots" directly under the apertures in the gas delivery pipes.

October

Densitometric curves at the end of the growing season are very similar to those obtained from August data. Sample variability was dampened somewhat due to the more uniform maturity stages of plant groups. Again, the color content, especially red, was significantly reduced by SO_2.

Regression Analysis

Simple linear regression equations and coefficients were calculated for each color and for total density for all dates and treatments (Table 22.3). The ratios between red and green densities also were computed. The SO_2 rates used in these calculations were cumulative means from the beginning of the growing season until the time of photography. Values are presented in Table 22.1.

In June, a highly significant linear depression of red density was observed across treatments; no other trends were significant. On the other hand, ZAPS II data were significantly or highly significantly linear for all colors and for total density.

In August, red density changes were not significant on ZAPS I, but blue, green, and total were. No significance was obtained on ZAPS II, clearly because of the high variation associated with samples.

By October, the only significant linear relationship between color and fumigation on ZAPS I was in the red wave lengths. ZAPS II showed a significant red/green ratio. This was due to the relatively faster loss of green than red color with fumigation. The biological significance of this observation is unclear. In general, the insensitivity of the red/green ratio to vegetational changes was disappointing. Research by Schott and others (Schott *et al.*, ND) has suggested that this ratio is a more sensitive indicator of vegetational change than any single wave length range. Our data, admittedly derived somewhat differently, did not support this observation.

The densitometric analyses show that losses of color on infrared film occur due to SO_2 fumigation. Interplot variability causes some insensitivity of statistical tests, but at least high and control rates are significantly different in most cases.

The detection of such differences does nothing to explain them. We are therefore conducting field studies in 1979 to account for these reflectance differences on cellular and physiological levels. Chlorophyll contents of western wheatgrass leaves and microscopic sections for pathological signs will be collected and analyzed coincidentally with aerial photo missions.

Ground-Level Photography

The square-meter photo plots were mapped by individual species. These maps are filed for future comparisons, both between times of observation and scales of photography (i.e., aerial vs. ground imagery).

Densitometric readings from photo plots on Colorado State University's fertilizer study were provided to CSU for record and further analysis.

Aspect photographs recorded the visual differences in plant species reflectance across fumigation plots. Abrupt density changes at treatment boundaries also were conspicuous. The principal value of these photographs is as visual references. They are not suitable for either densitometric analyses or mapping.

TABLE 22.3. LINEAR REGRESSION EQUATIONS ESTIMATING PHOTOMETRIC DENSITY (Y) AS A FUNCTION OF SULFUR DIOXIDE FUMIGATION (X)

ZAPS I				ZAPS II			
DATE	COLOR	EQUATION	r^2	DATE	COLOR	EQUATION	r^2
JUNE 1978	RED	Y = .5100-.0097X	.99**	JUNE 1978	RED	Y = .4529-.0168X	.93*
	GREEN	Y = .5865-.0204X	.80		GREEN	Y = .8051-.034X	.95*
	BLUE	Y = .9486-.027X	.86		BLUE	Y = 1.2283-.056X	.98**
	TOTAL	Y = .5111-.0165X	.85		TOTAL	Y = .6061-.0254X	.99**
	R/G	Y = .8636-.019X	.49		R/G	Y = .5584-.0054X	.16
AUGUST	RED	Y = 1.2538-.0341X	.82	AUGUST	RED	Y = 1.0522-.0309X	.79
	GREEN	Y = .7853-.0217X	.93*		GREEN	Y = .7754-.0161X	.45
	BLUE	Y = 1.7056-.0433X	.91*		BLUE	Y = 1.6810-.0431X	.71
	TOTAL	Y = .8709-.0273X	.92*		TOTAL	Y = .8023-.0195X	.61
	R/G	Y = 1.5924+.0019X	.009		R/G	Y = 1.3651-.0144	.63
OCTOBER	RED	Y = .8505-.0277X	.95*	OCTOBER	RED	Y = .6788-.0222X	.76
	GREEN	Y = .6190-.0197X	.78		GREEN	Y = .6043-.0294X	.79
	BLUE	Y = 1.0682-.0332X	.73		BLUE	Y = 1.0025-.0438X	.72
	TOTAL	Y = .6318-.0216X	.87		TOTAL	Y = .5650-.0253X	.78
	R/G	Y = $1.3711-8.5\text{X}10^{-5}$	$2.3\text{X}10^{-5}$		R/G	Y = 1.1158-.0297	.96*

* $P \le .05$
** $P \le .01$

CONCLUSIONS

The large scale (1:3,700) aerial photographs were subjected to densitometric analysis. A general reduction in density with increased SO_2 was observed with red, green, and blue wave lengths. Significant differences were observed between control and high SO_2 levels at most dates and for most colors. Total density changes were less consistent, as were red/green density ratios.

Absolute density values differed among seasons and between locations due to several confounded variables. These included inherent inter- and intraplot variation, differences in species composition and cover, and differing phenologic rates. They were further influenced by differences in photographic conditions, exposure, and processing.

Within dates and treatments, photographic density differences, both observed and measured, were clearly related to SO_2 fumigation.

Linear regression analyses quantified the direct relationship between SO_2 and color loss in vegetation. The red density, representing near-infrared reflecting species and objects, was depressed by SO_2, especially during the active growth season.

REFERENCES

Schott, J. R., D. W. Gaucher, and J. E. Walker, ND. Aerial Photographic Technique for Measuring Vegetation Stress from Sulfur Dioxide. Calspan Corp. Rep. YB-5967-M-1.

Taylor, J. E., W. C. Leininger, and R. J. Fuchs. 1976. Monitoring Plant Community Changes due to Emission from Fossil Fuel Power Plants in Eastern Montana. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana, June, 1975. R. A. Lewis, N. R. Glass, and A. S. LeFohn, eds. EPA/600-3-76-013, U. S. Environmental Protection Agency, Corvallis, Oregon. pp 14-40.

Woodcock, W.E. 1976. Aerial Reconnaissance and Photogrammetry with Small Cameras. Photogrammetric Eng. and Remote Sensing. 42:503-511.

SECTION 23

SIMULATION OF A GRASSLAND SULFUR CYCLE

M. B. Coughenour, W. J. Parton, W. K. Lauenroth,
J. L. Dodd, and R. G. Woodmansee

ABSTRACT

A simulation model was developed as a conceptual tool to organize information on sulfur cycling in a grassland ecosystem. The model successfully simulated the concentrations of sulfur in shoots of two plant functional groups over the course of two growing seasons and seemed to realistically represent such processes as microbial mineralization-immobilization, sulfate adsorption by soil, and nematode consumption. The greatest transfers of sulfur in the system were between soil sulfate and microorganisms. Various turnover rates were calculated, ranging from $3 \chi 10^{-5}$ yr^{-1}, for the most resistant components of humus, to 10 yr^{-1}, for the highly labile metabolic litter component. Only 2-4% of the total system flux of sulfur was reapportioned among system components, indicating that the model system was near equilibrium.

INTRODUCTION

Man's activities seemingly play an important role in the availability of sulfur in both natural and agricultural ecosystems. Fossil-fuel combustion adds millions of tons of sulfur to the biosphere annually (Kellog *et al.*, 1972), yet sulfur deficiencies in agro-ecosystems are becoming increasingly widespread because of the use of low-sulfur NPK fertilizers in high-yield agricultural practices (Bell, 1975) and the increasing emphasis on air-pollution control. We will present here a method to assess the effects of anthropogenic activities on nutrient cycling, using simulation modeling to integrate information, concepts, and assumptions about sulfur transformations in ecosystems.

The cycling of sulfur in grazed pastures was first elucidated by Walker (1957), who showed that the dynamics of the sulfur cycle are particularly important in pastures where nitrogen is continually added through fixation and in ungrazed harvested pastures where sulfur is not returned to soil in urine. Because responses in animal productivity to sulfur fertilizers depend on the integrated processes of plant uptake, animal utilization, animal return, and microbial release, subsequent studies were initiated, which led to the formulation of a kinetic mathematical model of the sulfur cycle. These studies revealed that the productive potential of the system depends on the rates of sulfur transfer through, rather than the quantities present in, any given system component (May *et al.*, 1968; Till and May, 1970, 1971; May *et al.*, 1972).

Our model provides a similar dynamic framework for understanding the nature of sulfur cycling in a grassland ecosystem dominated by western wheatgrass (*Agropyron smithii*) in southeastern Montana (Heitschmidt *et al.*, 1978). We feel the model is conceptually sound because the information it contains was either drawn from the literature or based on experience when published information was lacking or inappropriate. The model in its present form will be more useful as a conceptual than as a predictive tool. The model was tested by comparing its behavior with limited field data from the actual ecosystem.

Model Overview

Notation

f_j	jth flow of sulfur ($g\ S \cdot m^{-2} \cdot day^{-1}$) associated with an F_j flow of carbon (biomass)
S	sulfur state variable ($g\ S \cdot m^{-2}$)
C	carbon state variable ($g\ C \cdot m^{-2}$)
CS	C/S ratio
SC	S/C ratio
a_j	rate coefficient used to calculate sulfur flow f_j
E(x)	flow rate reduction factor (effect) as a function of variable x
V	maximum rate

The sulfur-cycle submodel (Figure 23.1) should not be viewed as a set of independent calculations, because it relies extensively on information supplied by other simultaneously executed submodels describing primary producer-carbon cycling (Detling *et al.*, 1979), decomposer-carbon and nitrogen cycling (McGill *et al.*, 1978), and abiotic processes (Parton, 1978). Consequently, sulfur state variables have names similar or identical to those of the other submodels. The assumption here is that there is a functional correspondence between elements (C,N,S) in all organism-mediated flows of biomass or substrates.

Although the producer-carbon submodel individually simulates roots and rhizomes (or crowns), these have been grouped into the single category

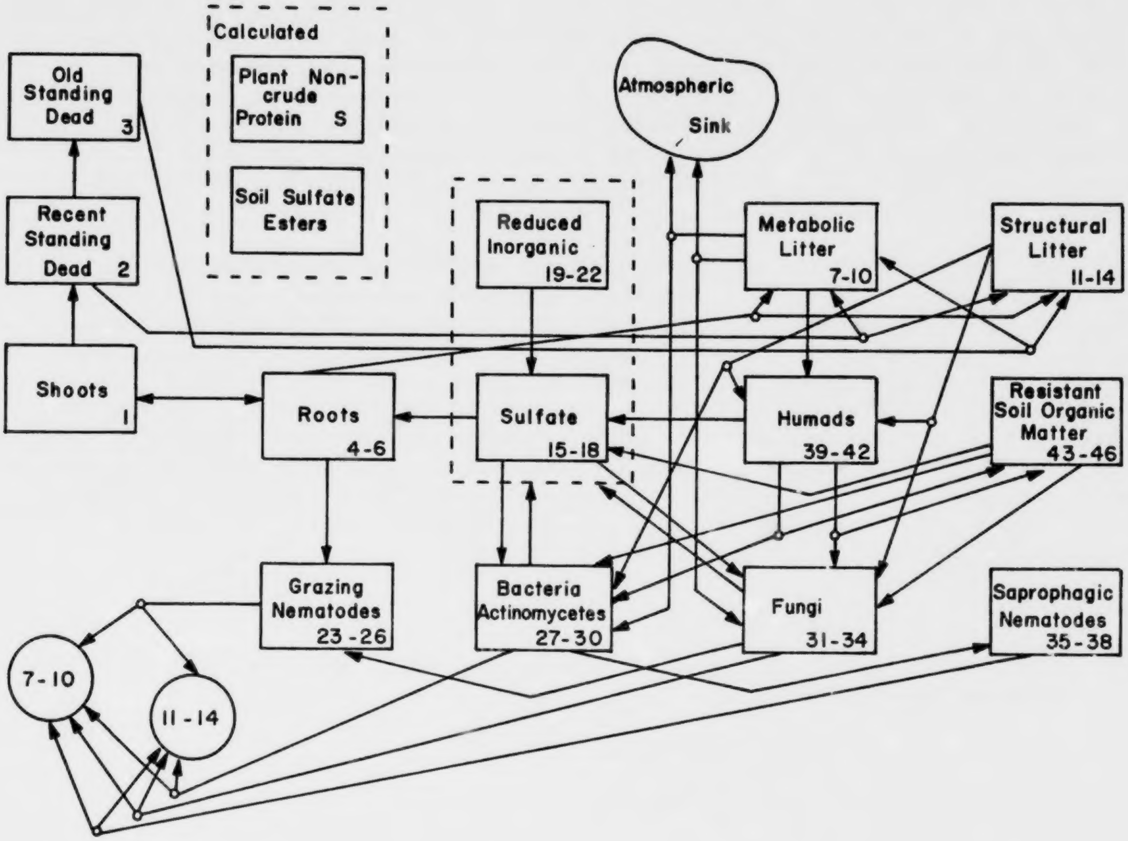

Calculated
Plant Non-crude Protein S
Soil Sulfate Esters
Old Standing Dead 3
Recent Standing Dead 2
Shoots 1
Roots 4-6
Atmospheric Sink
Reduced Inorganic 19-22
Sulfate 15-18
Metabolic Litter 7-10
Structural Litter 11-14
Humads 39-42
Resistant Soil Organic Matter 43-46
Grazing Nematodes 23-26
Bacteria Actinomycetes 27-30
Fungi 31-34
Saprophagic Nematodes 35-38
7-10
11-14

Figure 23.1. Flow diagram showing structure of sulfur cycle model. Large circles with numbers are duplicate representations of metabolic and structural litter. Small circles represent the partitioning of a flow. Flows to the atmospheric sink (violatilization) are assumed to return immediately to metabolic litter.

"roots" for the sulfur submodel. Likewise, young leaves, mature leaves, and stem components of the producer model have been grouped as "shoots" for the sulfur submodel. Roots are simulated in three soil strata: 0-5, 5-20, and 20-60 cm; and producers are simulated in two functional groups: *Agropyron smithii* and "other" species.

A considerable degree of correspondence exists between the decomposer-nitrogen and sulfur submodels. All three elements exactly correspond for bacteria, fungi, litter, humads, and resistant organic materials. The decomposer model is especially useful for calculating sulfur flows because the microbes themselves are the decomposers. The relationship between microbial demands and substrate C/S ratios controls mineralization-immobilization. Furthermore, in the model the substrate can be "altered" in quality and nutrient content during decomposition by simulating humads (discussed below) and the metabolic and structural components of litter, roughly analagous to the labile and resistant components of Hunt (1977). Thus carbon, nitrogen, and sulfur mineralizations are not strictly parallel processes. Sulfur mineralized early can be recycled to microbes for later use (Stotzky and Norman, 1961), such as during decomposition of more resistant materials. If there is an imbalance between N and S relative to microbial needs, the deficient element is immobilized.

The metabolic fraction of litter is soluble, high in nitrogen, and rapidly decomposable; while the structural fraction is insoluble, low in nitrogen and slowly decomposable. Only decomposition of structural materials is affected by microbial C/N and C/S ratios. The term humads was coined by McGill *et al.* (1978) by blending the words humus and adsorb. Humads are that component of soil organic material having a relatively short turnover time and are formed in a little-understood fusion-adsorption relationship between soluble and insoluble litter fractions and clay minerals. Resistant soil organic matter is humus having an extremely slow turnover rate. Bacteria and fungi were treated separately because they have different responses to abiotic variables and abilities to decompose soluble and insoluble substrates. All belowground components except roots were simulated at four depth strata: 0-1, 1-5, 5-20, and 20-60 cm. The top layer is a receptacle for litter from aboveground plant materials. Some physical mixing with soil is assumed.

The model was programmed in the SIMCOMP simulation language (Gustafson and Innis, 1973) version 4.0 (Gustafson, 1977). Daily maximum and minimum air temperatures, precipitation, humidity, and cloud cover are driving variables for the abiotic submodel, which in turn calculates temperatures and moisture levels at any point in the soil profile. Simulation proceeds over a daily time step. Model dynamics were tested against field data from the Taylor Creek Site in southeastern Montana (Dodd *et al.*, 1978) for the period 20 April 1975 through 23 September 1976.

Soil Sulfur

Inorganic sulfur is either completely oxidized as sulfate or found in a number of lower oxidation states, the most important of which is sulfide. Sulfate levels may range from 1 to 45 ppm in soils (Evans and Rost, 1945). With adequate aeration the sulfide form is usually present at only 1-3 ppm (Freney, 1961). Sulfate is found in water-soluble, adsorbed, or insoluble forms in soils. Free gypsum ($CaSO_4$) in the lower parts of semi-arid soils is common (Williams, 1975).

The model distinguishes sulfate in all three forms, but only one state variable is shown for clarity (Figure 23.1). Although inorganic sulfur has numerous oxidation states below that of sulfate, for our purposes we can consider them all as "reduced" and assume that the predominant form is S^{-2}.

Organic sulfur can be extracted in two distinguishable fractions from soil: hydriodic acid reducible (HI) and carbon bonded (Freney, 1961; Freney *et al.*, 1971). Hydriodic acid-reducible sulfur are sulfate esters and usually constitutes about 50% of total organic sulfur. The origin of these esters is uncertain, but they may be simply the products of the metabolism of 3'-phosphoadenylyl sulfate (PAPS) by plants and microorganisms:

$$\text{XOH} + \text{PAPS} \xrightarrow{\text{sulfokinase}} \text{PAP} + \text{XOSO}_3^- \qquad (1)$$

where XOH is any of a number of acceptor molecules and PAP is the desulfated phosphoadenylyl (Peck, 1969). Carbon-bonded sulfur probably originates from sulfur-containing amino acids. Because they are rapidly decomposed, free amino acids do not normally accumulate in soils; but the oxidized forms, cysteic acid and methionine sulfone, accounted for 21% of the total sulfur and 45% of the carbon-bonded sulfur in a virgin grassland soil (Freney *et al.*, 1972).

Ideally, the sulfur submodel would include separate state variables for carbon-bonded and esterified sulfur and the appropriate transformations for each. However, lack of information prohibits including such complexity in the model, so HI sulfur can be calculated best with Eq. 10. The model assumes that both organic sulfur forms are present in humads and resistant soil organic material.

Microbial Processes

Sulfur transformations in soils are mainly microbiological (Freney, 1967) and are of four types: biosynthetic sulfate reduction, respiratory sulfate reduction, respiratory sulfur oxidation, and photosynthetic sulfur oxidation (Peck, 1975). Respiratory sulfate reduction is unimportant in aerobic environments (Starkey, 1950), as it is inhibited by oxygen (Peck, 1975). Photosynthetic oxidation can be disregarded except in consistently anaerobic conditions. Obligate autotrophic respiratory oxidizers are important only in soils with added elemental sulfur, since sulfur can be oxidized in most soils by common heterotrophic microorganisms (Starkey, 1950).

The normal range of sulfur concentrations in microorganisms is 0.1% to 1.0% of the dry weight (Alexander, 1971). Additional information about microbial chemical composition was used in a detailed calculation of the C/S ratios of structural and metabolic components of bacteria and fungi (Coughenour, 1978). The structural component includes cell walls and associated membrane lipoproteins, while the metabolic component includes all soluble intra-cellular material. If structural composition is fixed, metabolic sulfur concentration is free to vary according to the variable cell sulfur status. The calculated C/S ratios for structural components were 335 for fungi and 210 for bacteria. Bacterial metabolic C/S ratios range from 35 to 75, yielding total cell C/S ratios from 50 to 100. Fungal metabolic C/S ranges from 120 to 250, yielding total cell C/S ratios from 150 to 280.

When microbial death is calculated in the decomposer submodel, sulfur flow is controlled by carbon flow:

$$f_1 = \frac{F_1}{CST_m} \qquad (2)$$

where f_1 and F_1 are the flows ($g \cdot m^{-2} \cdot day^{-1}$) of structural sulfur and carbon due to death, respectively, and CST_m is the fixed C/S ratio of the microbial structural component. The flow from metabolic components varies with the C/S ratio of the microbes:

$$f_2 = \frac{F_2}{CS_m} - f_1 \qquad (3)$$

where f_2 and F_2 are the flows of metabolic sulfur and carbon due to death, and CS_m is the C/S ratio of the microbes.

Sulfate in solution is available for microbial uptake, but little is known about microbial uptake kinetics. For a number of aerobic aquatic bacteria, uptake increases with sulfate concentration up to a maximum rate. Increasing concentrations of glucose in the media may increase uptake, while increasing cysteine or methionine in the media decreases sulfate uptake (Monheimer, 1975). Binding of labeled sulfate by *Thiobacillus ferroxidans* follows typical saturation kinetics, but cysteine and methionine do not affect binding (Tuovinen *et al.*, 1975), as would be expected in view of the role of sulfate in autotrophic metabolism. Thus, we assumed that sulfate is taken up from solution according to Michaelis-Menten enzyme kinetics: uptake is curtailed if sulfur demands are met by organic substrates, and uptake is enhanced by greater rates of carbon utilization.

Sulfate uptake rate is therefore modified by the effects of soil water and temperature on general microbial metabolism and by the demand for sulfur as expressed in the microbial C/S ratio:

$$a_3 = E_m(T) \cdot E_m(W) \cdot E_u(CS_m) \qquad (4)$$

and

$$f_3 = a_3 \cdot C_m \cdot \frac{V_{max} \cdot [SO_4]}{K_m + [SO_4]} \qquad (5)$$

where a_3 is the combined effect of temperature [$E_m(T)$, Appendix Figure 23.1-a], water [$E_m(W)$, Appendix Figure 23.1-b], and C/S ratio [$E_u(CS_m)$, Appendix Figure 23.1-c]; V_{max} is the maximum uptake rate; $[SO_4]$ is the concentration of sulfate in soil solution (ppm S); C_m is the microbial biomass ($g\ C \cdot m^{-2}$); and K_m is the half-saturation constant. First approximations of V_{max} and K_m values were calculated from published data (Monheimer, 1975; Tuovinen *et al.*, 1975).

However, uptake rates were too great and microbial sulfur levels rose to excessively high levels and fluctuated erratically. This instability may have been because the simulation interval (1 day) was large relative to the much faster turnover rate of microbial sulfur implied by the literature rate constants. To maintain reasonable C/S ratios, we gave K_m a value of 250 ppm for bacteria and fungi and V_{max} values of 0.056 and 0.01 g S $\cdot$ day^{-1} $\cdot$ g^{-1} microbial C for bacteria and fungi, respectively.

During decomposition, some substrate sulfur is used to construct microbial biomass, while the excess is converted to inorganic forms (Freney, 1967). Thus, in the model the extent of mineralization-immobilization is affected by the relationship between the sulfur content of the substrate and the sulfur requirements of the microbes. Attempts to predict mineralization from substrate sulfur concentrations only have been variously successful (Barrow, 1960a, 1960b; Kowalenko and Lowe, 1975b; Freney and Spencer, 1960; Williams, 1967). Also, substrate C/S ratios may mean little unless the nature of the substrate molecule is considered (Barrow, 1961; Frederick *et al.*, 1957; Stewart *et al.*, 1966b).

During decomposition, a correlation between C, N, and S mineralizations is sometimes observed, but the strength of this correlation may vary during decomposition (Nelson, 1964; Kowalenko and Lowe, 1975b). Sulfur release is often better correlated with nitrogen mineralization than with CO_2 evolution (Kowalenko and Lowe, 1975b, Stewart *et al.*, 1966a). However, the mineralized N/S ratio varies, depending on which element is limiting (Stewart *et al.*, 1966a), and may not correspond to the substrate N/S ratio, simply because N and S do not occur in the same compounds or substrate fractions (Freney and Stevenson, 1966). To avoid these difficulties, we considered each element separately in the model, microbial activity explicitly, and four substrate types.

It is difficult to ascertain whether sulfate or sulfide is the form directly mineralized from decomposing organic materials. When aeration was varied by changing soil moisture, no detectable levels of sulfide were found until 80% field capacity was reached (Chaudry and Cornfield, 1967). Very little sulfide was mineralized from cysteine, suggesting either that oxidation of sulfide to sulfate is very rapid or that sulfide is indeed not an intermediate in sulfate production (Freney, 1960). Decomposition of cysteine or cystine by single-species cultures produces either sulfate or reduced forms, depending on the species. Because of species interactions, mixed populations generally produce only sulfate under aerobic conditions (Freney, 1967). Either sulfate or methyl mercaptan and dimethyl disulfide are formed when methionine is decomposed aerobically by mixed populations (Frederick *et al.*, 1957; Freney, 1967). The last two compounds are volatile and may escape from the soil. Soils treated with a variety of sulfur-containing organics evolve methyl mercaptan, dimethyl sulfide, dimethyl disulfide, carbonyl sulfide, and carbon disulfide. Under aerobic conditions, 1.9% and 3.0% of total added sulfur are volatilized from dried and fresh plant materials, while under anaerobic conditions the percentages rise to 2.5% and 3.3% (Banwart and Bremner, 1976).

In the model, mineralization and sulfate uptake are affected by the same temperature and moisture functions. The model also includes an effect of microbial C/S that mirrors the C/S effect on sulfate uptake:

$$a_4 = E_m(T) \cdot E_m(W) \cdot E_{mn}(CS_m) \qquad (6)$$

$$f_4 = a_4 \cdot S_m \cdot V_{mn} \qquad (7)$$

where $E_m(T)$ and $E_m(W)$ are as in Eq. 4, $E_{mn}(CS_m)$ is the effect of C/S (Appendix Figure 23.1-d), a_4 is the combination of these effects, f_4 is the mineralization rate (g S · m^{-2} · day^{-1}), V_{mn} is the maximum rate (day^{-1}), and S_m is microbial sulfur (g S · m^{-2}). Maximum rates chosen were 0.8 day^{-1} for bacteria and 0.165 day^{-1} for fungi. The product of mineralization is sulfate or sulfide, depending on aeration. Sulfate is the sole product until 80% field capacity is reached, then the sulfate percentage decreases linearly to 0% at 133% field capacity (Appendix Figure 23.1-e).

The oxidation of sulfide to sulfate is controlled by soil aeration and bacterial metabolism. Bacterial metabolism is expressed as a function of temperature and water. The oxidation relationship is:

$$a_5 = E_m(T) \cdot E_m(W) \cdot E_{ox}(W) \qquad (8)$$

$$f_5 = a_5 \cdot V_{ox} \cdot S_R \qquad (9)$$

where $E_m(T)$ and $E_m(W)$ are the effects of temperature and water in bacteria; $E_{ox}(W)$ is the effect of water on aeration (Appendix Figure 23.1-e); a_5 is the combined effect; f_5 the oxidation rate (g S · m^2 · day^{-1}); S_R is the quantity of reduced forms (g S · m^{-2}); and V_{ox} is the maximum rate (day^{-1}), based on results from a silt loam soil in which 55% of the reduced sulfur was oxidized in 3 weeks (Attoe and Olsen, 1966). This expression thus assumes that oxidation is proportional to S_R.

Volatilization of sulfur during decomposition is represented as a proportion of metabolic litter decomposition, increasing slightly as aeration declines:

$$f_6 = F_6 \cdot P_v(W) \cdot SC_{me}$$

where f_6 is volatilization (g S · m^{-2} · day^{-1}); F_6 is metabolic litter decomposition (g C · m^{-2} · day^{-1}); $P_v(W)$ is the variable proportion as a function of soil water (Appendix Figure 23.1-h), based on data of Banwart and Bremner (1976); and SC_{me} is the S/C of metabolic litter. We assume that this equation applies to all soil layers.

Because of the uncertainty of their origin, sulfate esters are not explicitly treated but are assumed to be associated with humads and resistant soil organic matter. A regression equation based on data of Cooper (1972) and Bettany *et al.* (1973, 1974) predicts that HI sulfur (S_{HI}, g S · m^{-2}) is a function of total sulfur (S_{TOTAL}, g S · m^{-2}) and total carbon (C_{TOTAL}, g C · m^{-2}):

$$S_{HI} = 0.71 \cdot S_{TOTAL} - 0.0029 \cdot C_{TOTAL} \qquad (10)$$

This equation can be used to calculate HI sulfur at any point in the simulation.

Sulfate may be released through the action of sulfatase enzymes on soil sulfate esters:

$$ROSO_3^- + H_2O \rightarrow ROH + H^+ + SO_4^= \qquad (11)$$

Sulfatase has been detected in plants, animals, and microorganisms. Arylsulfatase activity in soils can be measured by the method of Tabatabai and Bremner (1970), while the Houghton and Rose (1976) method can be applied to a variety of sulfatases. Arylsulfatase activity correlates well with total carbon and total sulfur and strongly with HI sulfur (Cooper, 1972). Activity is related to temperature (Tabatabai and Bremner, 1970) and soil water (Cooper, 1972). Sulfate may not be liberated directly from humic acids; rather, a sequential attack by depolymerizing and desulfating enzymes may be required (Houghton and Rose, 1976).

A regression equation, formulated with data of Tabatabai and Bremner (1970), predicts sulfatase activity as a function of total sulfur:

$$A = 2.026 \cdot (\text{ppm total soil sulfur}) \qquad (12)$$

where the zero-intercept was forced as a simplified assumption.

The units of A are µg p-nitrophenol · g^{-1} soil · hr^{-1}, since p-nitrophenol was the aryl product assayed. Knowing that one mole of sulfate is released for each mole of p-nitrophenol, we can make the conversion:

$$a_7 = 0.0011 \cdot S_{TOTAL} \qquad (13)$$

where a_7 is activity (g S · m^{-2} · day^{-1}), and S_{TOTAL} is total soil sulfur (g S · m^{-2}). This basic rate is then modified to reflect the hypothesis (Houghton and Rose, 1976) that depolymerizing enzymes must act on humic materials before sulfatase can take effect. Thus, the effects of water and temperature on microbial activity averaged for bacteria and fungi was included, assuming depolymerizing action is proportional to microbial activity. Furthermore, an effect of temperature on extracellular enzyme activity is incorporated, based on data of Tabatabai and Bremner (1970). The effect equals 1 (no effect) at 37°C, the standard temperature of their experimental procedure. The final expression is:

$$R = a_7 \cdot E_e(T) \cdot E_m(T) \cdot E_m(W) \qquad (14)$$

where a_7 is defined in equation 13, R is sulfur released (g S $\cdot$ m^{-2} $\cdot$ day^{-1}), $E_e(T)$ is the effect of temperature on extracellular enzymes (Appendix Figure 23.1-f), $E_m(T)$ and $E_m(W)$ are the average effects of temperature and water for the two microbial groups.

Because roots and microbes are the presumed source of the sulfatase enzyme, activity in deeper layers is scaled to that calculated for the surface layer according to differences in root and microbial biomass densities. If B_o = sum of root and microbial biomass per cm of soil in the surface layer, and B = the same measure in a deeper layer, then

$$\beta = \frac{B}{B_o} \qquad (15)$$

is the proportional decrease in activity for layer 1. Because sulfate esters are assumed to be associated with both humads and resistant soil organic matter, the total flow is divided between them in proportion to their relative sulfur concentrations:

$$\lambda = \frac{SC_H}{SC_R} \qquad (16)$$

$$\alpha = \frac{\lambda \cdot S_H}{\lambda \cdot S_H + S_R} \qquad (17)$$

$$f_7 = R \cdot \beta \cdot \alpha \qquad (18)$$

$$f_8 = R \cdot \beta \cdot (1 - \alpha) \qquad (19)$$

where λ is the lability of humad-associated esters compared to that of resistant organic-associated esters, SC_H and SC_R are the S/C ratios of humads and resistant organic material, α is the proportion of sulfate originating from humads, S_H is humad sulfur (g S $\cdot$ m^{-2}), S_R is resistant organic sulfur (g S $\cdot$ m^{-2}), f_7 is the sulfate release from humads (g S $\cdot$ m^{-2} $\cdot$ day^{-1}), and f_8 is the release from resistant organic material.

Decomposition rates of metabolic and structural litter, humads, and resistant organic material are calculated in the decomposer submodel, sulfur merely following carbon according to the C/S ratio of the substrate. As postulated, only the decomposition of structural litter components is influenced by sulfur. Because humads and metabolic components are considered sufficiently high in sulfur, their decomposers are not sulfur limited. When bacterial C/S is more than 68 and fungal C/S is more than 202, structural litter decomposition becomes limited; complete limitation is reached before the microbial sulfur content reaches minimum (Appendix Figure 23.1-g).

Plant Processes

Sulfate uptake by roots seems to follow classical Michaelis-Menten kinetics (Leggett and Epstein, 1956; Persson, 1969; Nissen, 1971). First, the sulfate undergoes a labile binding to positive sites in the apparent free space. Maintenance of these positive sites is likely related to root metabolism (Persson, 1969), but the quantity of ions reaching the sites seems to be affected also by a process mediated in the shoots, since decapitated plants bind less than nondecapitated plants (Ingelsten, 1966). The transfer of ions from the apparent free space to the xylem appears to be linked to the supply of energy-rich substances from the shoots rather than to a root-retention mechanism (Pettersson, 1966). Over a narrow range of media concentrations, uptake obeys a single set of Michaelis-Menten parameters (Leggett and Epstein, 1956). However, over a much broader range, uptake is mediated by a single multiphasic isotherm (Nissen, 1971) and passes through a series of phases, each separable from the next by a sharp concentration level. Michaelis-Menten kinetics apply within each phase, but V_m and K_m increase between phases as concentration rises. This mechanism is different from the dual high- and low-affinity mechanism postulated from nitrogen (Reuss and Innis, 1977) and phosphorous (Cole *et al.*, 1977).

Root sulfate uptake is calculated:

$$U = \frac{V_{max} \cdot [SO_4]}{K_m + [SO_4]} \tag{21}$$

$$f_9 = C_R \cdot E_R(T) \cdot E_R(W) \cdot E_u(CS_p) \cdot U \tag{22}$$

where U is the Michaelis-Menten basic uptake rate (g S $\cdot$ m^{-2} $\cdot$ day^{-1} $\cdot$ g^{-1} root carbon), V_{max} is the maximum rate, K_m the half-saturation constant, $[SO_4]$ is the solution sulfate concentration (ppm S), f_9 is uptake (g S $\cdot$ m^{-2} $\cdot$ day^{-1}), C_R is root carbon (g C $\cdot$ m^{-2}), $E_R(W)$ and E_R (T) are the effects of water and temperature on root metabolism (Appendix Figures 23.1-i and 23.1-j), and E_u (CS_p) is the effect of whole-plant S/C on uptake (Appendix Figure 23.1-k). Table 23.1 shows the V_{max} and K_m values used. Although K_m and switchpoint concentrations are those of Nissen (1971), his V_{max} values were reduced by 0.004 to match observed shoot-sulfur concentrations.

TABLE 23.1. V_{max} AND K_m PARAMETER VALUES USED IN THE MULTI-PHASE MICHAELIS-MENTEN EXPRESSION FOR ROOT UPTAKE*

SO_4 concentration (ppm S)	V_{max} (g S · m^{-2} · day^{-1} · g root $carbon^{-1}$)	K_m (ppm S)
< 1.088	3.04×10^{-6}	0.54
1.088/3.2	5.00×10^{-6}	1.60
3.2/12.8	7.48×10^{-6}	3.84
12.8/32.0	10.40×10^{-6}	9.92
32.0/80.0	17.80×10^{-6}	41.60
80.0/201.6	29.96×10^{-6}	124.80
201.6/768.0	77.80×10^{-6}	640.00
> 768.0	120.18×10^{-5}	20480.00

[*] V_{max} values are 0.4% those of Nissen's (1971), while K_m values are unmodified.

Movement from roots to shoots is also a metabolically mediated process (Pettersson, 1966; Ingelsten, 1966). Sulfur is freely mobile in the plant but is captured quickly in plant parts undergoing active cell division (Biddulph *et al.*, 1958). From the site of active uptake, sulfate moves rapidly to the xylem and thence to shoots. Inhibition by selenate on translocation therefore implies that inhibition is of whole-plant sulfate absorption rather than of root and shoot uptake separately, which corresponds to the inhibition of root uptake in decapitated plants (Ingelsten, 1966). Likewise, root uptake of nitrogen is not related to root-nitrogen content (Edwards and Barber, 1976). We therefore propose that sulfate uptake is related to whole-plant sulfur demand.

Sulfur is translocated to shoots in the model according to a predefined ratio of shoot S/C to root S/C, which is assumed in turn to be a function of plant phenology. This formulation has worked well in a model of nitrogen translocation (Reuss and Innis, 1977) and reflects observations of higher shoot-sulfur than root-sulfur contents in early phenophases.

Thus,

$$DSC = SC_{RT} \cdot I(Phen) \qquad (23)$$

$$f_{10} = F_{10} \cdot DSC \cdot \frac{S_1^{RT}}{S_{ROOT}} \qquad (24)$$

where DSC is the determined shoot S/C, SC_{RT} is actual root S/C, I(Phen) is the predefined ratio of shoot S/C to root S/C as a function of phenology (Appendix Figure 23.1-1), f_{10} is translocation from one root layer (g S $\cdot$ m^{-2} $\cdot$ day^{-1}), F_{10} is the daily shoot carbon growth increment (g C $\cdot$ m^{-2} $\cdot$ day^{-1}), SC_1^{RT} is the root sulfur in a layer and S_{ROOT} is root sulfur summed over all layers (g S $\cdot$ m^{-2}).

During death of plant parts, sulfur may be remobilized from the dying organs and thus conserved within the plant. We assume, however, that shoot death induced by freezing or drought will not conserve sulfur and postulate here that dying roots do not conserve sulfur. Sulfur follows carbon during non-sulfur-conserving death, with a metabolic/structural split determined by:

$$SM = S - \frac{\sigma \cdot C}{CST} \qquad (25)$$

$$CSM = \frac{(1 - \sigma) \cdot C}{SM} \qquad (26)$$

where SM is root or shoot metabolic sulfur (g S $\cdot$ m^{-2}), S is root or shoot total sulfur, C is root or shoot carbon (g C $\cdot$ m^{-2}), σ is the fraction of structural carbon in dying tissue, CST is the fixed C/S ratio of structural components, and CSM is the variable C/S of dying metabolic components. As calculated from available information on plant chemical composition, CST was 63 for shoots and 1030 for roots (Coughenour, 1978).

During shoot senescence, sulfur is remobilized in the plant by protein hydrolysis in senescing cells (Williams, 1955). This remobilized sulfur is probably transferred to belowground organs, where it may be utilized the following season during shoot regrowth, as is nitrogen (Clark, 1977). However, net export of sulfur from Sudan grass leaves is delayed and depressed more than that of nitrogen (Barrien and Wood, 1939, cited in Williams, 1955). Evidence suggests that sulfur is conserved in these grasses, as shoot sulfur in *A. smithii* declined from 0.12%, in live shoots, on 1 August 1975 to 0.08%, in recent dead, on 1 September to 0.06%, in old dead, the following year.

For sulfur-conserving shoot death,

$$CS_S = (CN_S + \delta) \cdot NS_S \tag{27}$$

$$f_{11} = \frac{F_{11}}{CS_S} - \frac{F_{11} \cdot \sigma}{CST_S} \tag{28}$$

$$CSM_S = \frac{(1 - \sigma) \cdot F_{11}}{f_{11}} \tag{29}$$

where CS_S is the C/S of senescing shoots after conservation, CN_S is the C/N ratio of shoots, δ is the increase in C/N in tissues where protein hydrolysis has occurred, σ is the fraction of structural shoot carbon, NS_S is the N/S of shoots, F_{11} is total shoot-carbon death ($g\ C \cdot m^{-2} \cdot day^{-1}$), f_{11} is total metabolic sulfur in dying shoots ($g\ S \cdot m^{-2} \cdot day^{-1}$), CST_S is the fixed shoot structural C/S, and CSM_S is the C/S of dying metabolic components of dying shoots. Thus, structural sulfur in dying shoots is:

$$f_{12} = \frac{F_{11}}{CS_S} \quad f_{11} \tag{30}$$

and total remobilization of sulfur is:

$$f_{13} = F_{11} \cdot (1 - \sigma) \cdot \left(\frac{1}{CSMD_S} - \frac{1}{CSM_S}\right) \tag{31}$$

which is distributed among roots in all layers; $CSMD_S$ is the C/S of the metabolic component before protein hydrolysis.

The effect of sulfur on shoot growth and photosynthesis is critical to any sulfur cycling submodel; however, scant information on the process which is distributed among roots in all layers; $CSMD_S$ is the C/S of the metabolic component before protein hydrolysis.

The effect of sulfur on shoot growth and photosynthesis is critical to any sulfur-cycling submodel; however, scant information on the process exists, especially for these specific grasses. Even though concentrations in *A. smithii* shoots are typically about 0.1% S, well below the critical range of 0.3-0.35% supposedly required for maximum yield (Metson, 1973), growth does not seem impaired (Dodd *et al.* 1978). Another index of sulfur status is the N/S ratio. If a constant protein N/S ratio is assumed, non-protein nitrogen should accumulate, yielding a high N/S ratio when sulfur is deficient. The critical N/S ratio for grass is thought to be 15-20 (Metson, 1973). Typically, however, *A. smithii* N/S is 20-25 in early, and 10-15 in late, phenophases. This phenological change makes interpretation of N/S ratios difficult. When nitrogen is limiting, as is likely here, N/S ratios may mean little. Until more satisfactory information is provided, we propose that shoot metabolism is affected only if nitrogen is non-limiting (above 2.5% N for photosynthesis and 2.0% N for growth). When N is non-limiting, photosynthesis and growth are not sulfur limited at $N/S \leq 25$, but will decline linearly to complete restriction at $N/S = 35$.

Nematodes

A model of plant parasitic nematode population dynamics (Ferris, 1976) was adapted as a driving model for carbon, nitrogen, and sulfur flows. Although the model was intended for *Meloidogyne* nematodes, which host on grapevine, functions of soil water and temperature seemed comparable to observations for nematode groups found in South Dakota (Smolik, 1975). Therefore, general principles of population growth should be roughly applicable here. Population estimates and biomass conversion values determined by J. Smolik for the Taylor Creek Site (Coughenour, 1978) were used to calculate initial conditions and to roughly verify model performance.

The primary departure from Ferris' (1976) model is a consequence of dormancy, which allows nematodes in Montana to withstand conditions of severe stress not found in the grapevine system. To represent this process, we allowed nematodes to pass between active and dormant stages at specified critical soil moisture (8% field capacity) and temperature (2.5°C) levels.

To calculate consumption as a function of active population size, we used Santmeyer's (1956) data to determine metabolic rate as a function of temperature. The first approximation to intake is:

$$I_c = M_r(T) \cdot 4.8 \frac{\text{cal}}{\text{ml } O_2} \div 4500 \frac{\text{cal}}{\text{g food}} \cdot$$

$$2.0 \text{ activity requirement} \div 0.5 \text{ assimilation efficiency} \cdot$$

$$0.25 \frac{\text{g dwt}}{\text{g fwt}} \cdot 24 \frac{\text{hr}}{\text{day}} \div 35 \frac{\text{g C}}{\text{g dwt}} = M_r(T) \cdot 0.02925 \qquad (32)$$

(Smolik, 1974), where I_c is intake (g C $\cdot$ g^{-1} nematode C $\cdot$ day^{-1}), fwt and dwt are fresh and dry weights, and $M_r(T)$ is the temperature-dependent metabolic rate (ml O_2 $\cdot$ g^{-1} fwt $\cdot$ hr^{-1}), shown in Appendix Figure 23.1-m. Respiration is found:

$$R = AE \cdot I_c \qquad (33)$$

where R is respiration (g C $\cdot$ g^{-1} nematode C $\cdot$ day^{-1}), AE is assimilation efficiency (0.5), and I_c is intake. The population model calculates growth in terms of numbers but not of biomass, hence the following algorithm. If N is nematode biomass before growth and death increments, if N_B is nematode biomass after death and growth increments, and if N_x is nematode biomass corrected for death (D) but not growth, then

$$N_x = N - D \tag{34a}$$

$$N_B = N - D + G \tag{34b}$$

$$G = N_B - N_x \tag{35}$$

where G is the biomass growth increment ($g\ C \cdot m^{-2} \cdot day^{-1}$) caused by such processes as hatching, moulting, and egg laying. Intake is reapproximated to account for the growth increments:

$$I_c = \frac{G + R}{AE} \tag{36}$$

and feces production follows as

$$E = I_c \cdot (1 - AE) \tag{37}$$

(Petrusewicz and Macfadyen, 1970).

Intake of nitrogen and sulfur follows carbon according to the ratios in the food. The N and S requirements for growth are:

$$RQ_n = G \cdot NC_{nem} \tag{38}$$

$$RQ_s = G \cdot SC_{nem} \tag{39}$$

where RQ_n and RQ_s are the growth requirements ($g\ N \cdot m^{-2} \cdot day^{-1}$, $g\ S \cdot m^{-2} \cdot day^{-1}$), G is carbon growth ($g\ C \cdot m^{-2} \cdot day^{-1}$), and NC_{nem} and SC_{nem} are the N/C and S/C ratios of nematodes. Nematode composition was calculated as C/N/S = 46/14/1 (Coughenour, 1978). Thus, N and S in excess of requirements are determined:

$$E_n = I_n - RQ_n \tag{40}$$

$$E_s = I_s - RQ_s \tag{41}$$

where I_n and I_s are total N and S consumptions. Ammonium accounts for 27-71% of total nitrogen excretion (Rogers, 1969) and will be assumed here to be constant at 50%. Excreted carbon and sulfur are assumed to flow from microbivores to metabolic and structural litter in a 1/3 ratio, while all C, N, and S excreted by herbivores will flow to metabolic litter, since they consume only the metabolic fraction of root cells.

Abiotic Processes

Soil sulfate adsorption-desorption is formulated with the Freundlich isotherm:

$$\Delta S = b \cdot [SO_4]^{1/n} \qquad (42)$$

where $[SO_4]$ is solution sulfate concentration (ppm S), ΔS is the quantity adsorbed (g S $\cdot$ g^{-1} soil), b and n are empirical constants. The Freundlich isotherm is preferred to the Langmuir isotherm for sulfate because no adsorption maxima is found (Harward *et al.*, 1962; Chao *et al.*, 1962) and increasing solution sulfate concentrations seem to activate additional adsorption sites (Barrow, 1967).

To determine b and n, we divided a 60-cm soil core into four strata (0.5, 5-10, 10-20, and 20-60 cm) for the following analysis. Five grams of soil were shaken for 24 hours in a 10/1 solution to soil ratio. Solution sulfate concentrations were 0, 5, 10, 25, 50, 100, 200, 300, and 400 ppm S. The quantity adsorbed was the difference between solution concentrations before addition to soil and those after the shaking process. Results were fitted to the Freundlich equation by nonlinear least squares; the resulting parameters are shown in Table 23.2.

Successive approximation is used to implement the isotherm in the model. Initially, all sulfate is assumed to be in solution and the isotherm is used to determine ΔS for that sulfate concentration. The ΔS is used to determine a ratio of total adsorbed (g S $\cdot$ m^{-2}) to total soil (g S $\cdot$ m^{-2}) sulfate, which is then used to calculate a new value for the proportion in solution. This proportion yields a new approximation of solution concentration, and the cycle is repeated. Five or six iterations are sufficient for convergence on the equilibrium.

The solubility-product concept is applied to determine gypsum precipitation:

$$K_{sp} = [Ca^{+2}] \cdot [SO_4^{-2}] \cdot \gamma^2 \qquad (43)$$

where K_{sp} is the solubility product (2.4×10^{-5} at 25°C; Tanji, 1969), and γ is the mean activity coefficient. When K_{sp} is reached, gypsum may precipitate (Dutt, 1964). Although K_{sp} is actually temperature dependent, this feature is not included in the model. The concentration of gypsum can be estimated:

$$CaSO_4 = \frac{[Ca^{+2}] \cdot [SO_4^{-2}] \cdot \gamma}{K_D} \qquad (44)$$

TABLE 23.2. FREUDLICH ISOTHERM PARAMETERS FOR TAYLOR CREEK SOIL*

Depth (cm)	b	$\frac{1}{n}$	Error mean Square of fit
0-5	4.88	0.361	187.8
5-10	4.50	0.281	106.7
10-20	5.74	0.365	354.0
20-60	1.74	0.634	51.4

*Data were fit to $\Delta P = b\ C^{1/n}$, where ΔP equals μg S adsorbed per gram soil and C equals ppm S in initial solution (μg S/ml H_2O).

where K_D is the dissociation constant ($4.9 \times 10.^{-3}$, Dutt 1964). The mean activity coefficient for the soil solution is estimated:

$$\ln \gamma = \frac{0.509\ z^2 \sqrt{u}}{1 + \sqrt{u}} \tag{45}$$

where z is the valence of the ion and u is ionic strength given by:

$$u = 1/2 \sum_{i=1}^{n} C_i\ z_i^2 \tag{46}$$

where C_i is the concentration of one of the n ions in solution (Dutt, 1962). The ions considered here include Na^+, K^+, Ca^{+2}, Mg^{+2}, and SO_4^{-2}. The solution concentration of cations are calculated with subroutine CATEX (Reiniger *et al.*, 1971; Frissel and Reiniger, 1974), which uses Vaneslow-like exchange equations to describe competition of the four cations for sites on the soil exchange complex. Cation exchange capacity and cation concentrations were obtained from a thorough chemical analysis of Taylor Creek soils (Coughenour, 1978).

The top soil layer is the receptacle of large quantities of above-ground litter, yet is only 1 cm thick. Soluble decomposition products are therefore assumed to move by mass flow to the next lower layer; for, as in a mixing cell, the solution concentration of a layer is uniform. The flow from the surface layer, then, is the product of total water drainage (supplied by the abiotic submodel) times the solution concentration of sulfate, metabolic litter, or desorbed humads. Because of the large solution interval (1 day), mass flow is restricted such that solution concentrations in the lower layer do not exceed those in the surface layer.

Diffusion of sulfate is described by Fick's Law, where the diffusion rate is proportional to the concentration gradient. Thus,

$$Df = D \cdot \frac{\Delta[SO_4]}{\Delta X} \cdot \theta \qquad (47)$$

where Df is diffusion rate (g S $\cdot$ cm^{-2} $\cdot$ day^{-1}), $\Delta[SO_4]$ is the concentration difference between layers, ΔX is the distance (cm) between midpoints of adjacent layers, D is diffusivity (cm^{-2} $\cdot$ day^{-1}), and θ is the volumetric soil water content, necessary here to express diffusion rate per cross-sectional area of soil.

Model Verification

Model performance was tested continually throughout its development with a 2-year data set from the Taylor Creek Site (Dodd *et al.*, 1978). The same data set was used for the final verification presented here. Because the sulfur submodel depends on the other submodels, their behaviors must be verified first.

The abiotic submodel was verified with soil-water data from each of four 15-cm layers to a 60-cm depth. Agreement with observed values was favorable in all layers over the 1975 season. Typically, soil water declines through the summer until replenished by fall rains. The most serious discrepancy was the underestimation of soil water at 0-15 cm in the spring of 1976 (Appendix Figure 23.2-a). This and other minor discrepancies could be related to variation in snowmelt and runoff or to field sampling problems (Parton *et al.*, 1978). Comparisons were more favorable by the end of the 1976 growing season in all layers, indicating a good approximation of the yearly water budget.

Comparisons between observed and simulated shoot-biomass dynamics were also satisfactory (Appendix Figures 23.2-c and .2-d). The timing and the magnitude of the peaks were well represented. The rapid growth phase from early to mid-season proved most difficult to simulate, probably because of the difficulty of distinguishing live from dead shoots during harvest measurements. Discrepancies during the death phase were less serious. The simulated dynamics for rhizomes (Appendix Figure 23.2-e). showed fewer fluctuations than the data. However, the May minimum-July maximum relationship was supported by the data. Strict verification of intraseasonal root dynamics was very difficult, if not impossible, again because of the lack of a reliable method for distinguishing live from dead roots in the field. The comparison here was between the sum of simulated live plus dead roots (belowground litter) and total root-biomass measurements from 0-10 cm soil cores (Appendix Figure 23.2-f). In view of this problem and the problem of large spatial sample variability, these comparisons seemed adequate.

The decomposer model was tested in part by the verification of root dynamics, since dead roots were included in belowground litter. A better test was to compare observed and simulated aboveground litter dynamics (Appendix Figure 23.2-g). Litter biomass is also affected by the transfer from standing dead to litter, a poorly understood process. This lack of understanding, along with the large variances in data, contributed to discrepancies between observed and predicted litter dynamics.

The simulated shoot-nitrogen concentrations compared favorably with observed values (Appendix Figure 23.2-h). The concentration dilution during growth showed the correct timing and magnitude except late in 1975, when dilution or remobilization was overestimated. Simulated live-shoot sulfur concentrations (Figures 23.2 and 23.3) agreed with data in 1975 for *A. smithii* and in 1976 for "other." The cause of the observed peak and decline in early August 1975 for *A. smithii* is unknown and was not matched by the model. This verification of shoot-sulfur concentrations establishes confidence in the formulation of translocation and root uptake. Simulation of standing dead-shoot sulfur concentrations (not shown) showed overestimations toward the ends of the seasons, indicating an underestimation of sulfur remobilization and retranslocation to roots.

RESULTS AND DISCUSSION

Total soil-sulfate levels ($g\ S \cdot m^{-2}$) are not presented, because they remained essentially constant throughout the simulation, reflecting a near balance of inputs and outputs to this central component. However, one important deviation should be noted. In late June 1975, sulfate levels dropped temporarily in the model by about $0.5\ g\ S \cdot m^{-2}$ at 1-5 cm, and by approximately $1.5\ g\ S \cdot m^{-2}$ from July through August 1975 at 5-20 cm. This behavior, caused by simulated precipitation of gypsum, suggested that the combined concentration of calcium and gypsum in the real system may be sufficiently high for this to occur. Further research is required to test this prediction, including a representation of soil-calcium removal from, and return to, the soil. The range of simulated sulfate-solution concentrations (Figure 23.4) was 16-328 ppm S. Highest levels coincided with periods of lowest soil moisture, towards the end of the growing seasons.

Sulfide or reduced inorganic sulfur (Figure 23.5) rose to maxima of 0.015 $g\ S \cdot m^{-2}$ in the 0-1 cm layer, 0.06 $g\ S \cdot m^{-2}$ in the 1-5 cm layer, 0.055 $g\ S \cdot m^{-2}$ in the 5-20 cm layer, and 0.09 $g\ S \cdot m^{-2}$ in the 20-60 cm layer. These levels correspond to 1.48, 1.10, 0.27, and 0.14 ppm S (soil basis). Although the initial conditions for this component were not known, levels later in the simulation indicated a balance between oxidation and reduction processes, implicit in the model formulation. The resulting low quantities of reduced forms suggested that autotrophic sulfur oxidizers may be insignificant in this ecosystem.

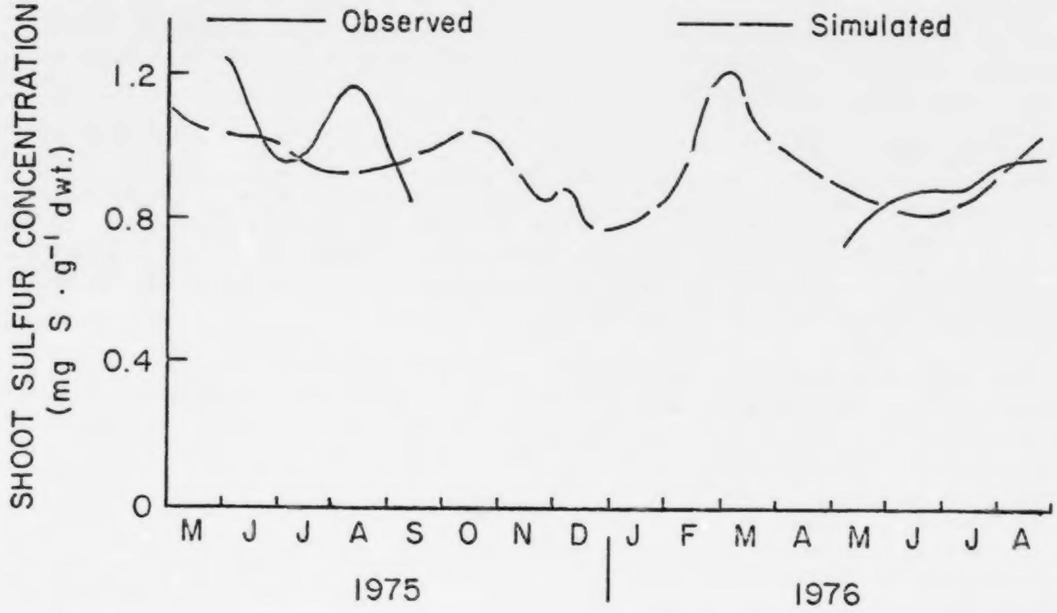


Figure 23.2. *A. smithii* live-shoot sulfur concentration verification.

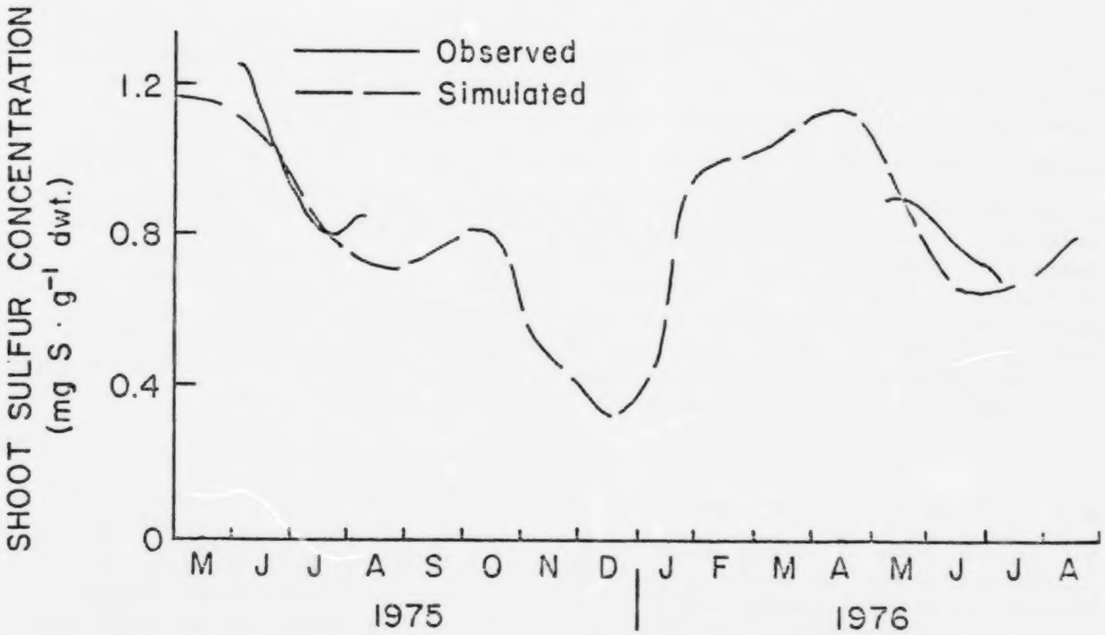


Figure 23.3. "Other" live-shoot sulfur concentration verification.

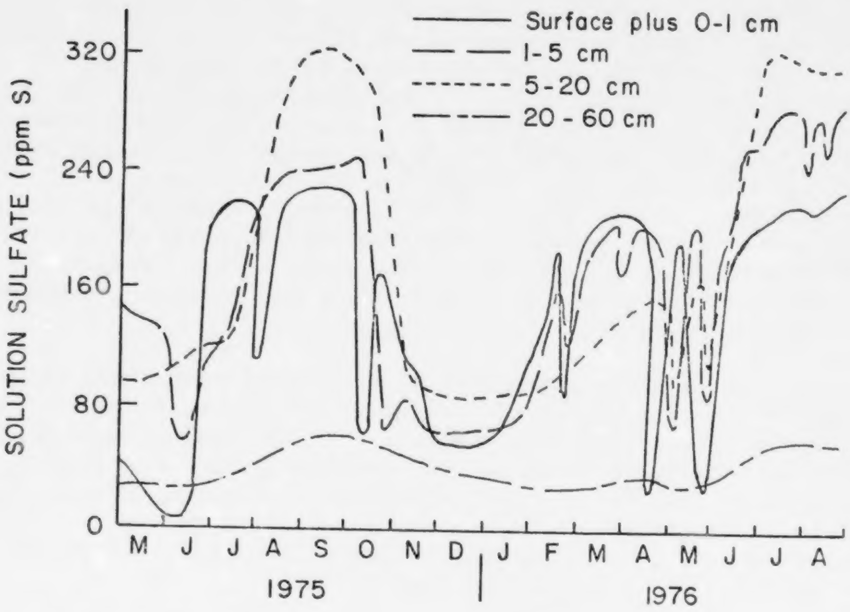


Figure 23.4. Sulfate-solution concentrations.

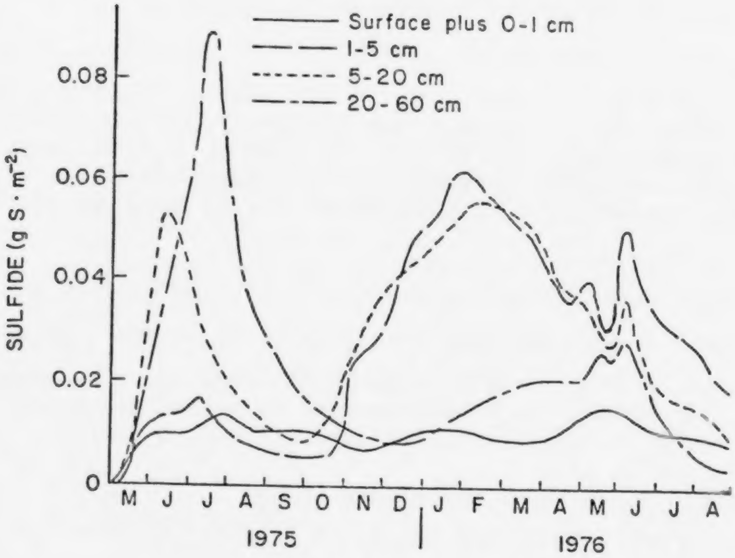


Figure 23.5. Sulfide or reduced inorganic sulfur.

The range of C/S ratios of bacteria and actinomycetes (Figure 23.6) was 57-85, while that of fungi (Figure 23.7) was 182-230, which were reasonable. Because bacterial C/S ratios exceeded 68 and fungal C/S ratios exceeded 202, sulfur limited the decomposition of structural litter components formulated in the model (Appendix Figure 23.2-g) The consistently wide C/S ratios of both groups at 20-60 cm were the result of low solution concentrations of sulfate in that layer. The narrowest C/S ratios generally coincided with high solution-sulfate concentrations. In the surface soil layer, widest C/S ratios were simulated in the spring of 1975, when conditions for microbial activity were most favorable, suggesting that sulfur recycling did not keep pace with rapid microbial growth and decomposition rates. Fungal C/S ratios fluctuated less than bacterial C/S ratios, probably because of the lower mineralization rate postulated for the fungi.

The range of sulfur concentrations of *A. smithii* live roots (Figure 23.8) was 0.13-0.25%, with concentrations declining during periods of root growth in both years, indicating a dilution effect. If the assumption that shoot-sulfur concentrations are coupled to root concentrations according to a phenologically dependent ratio holds, then the observed decline in shoot concentrations between years implies a similar decline in root concentrations. Because root uptake rates were adjusted to match this decline, root concentrations necessarily dropped between years.

Accumulated flows of sulfur over two periods of the simulation are presented in the form of input-output matrices (Tables 23.3 and 23.4). The 336-day period approximates the outcome of yearly processes, while the 182-day period reveals the outcome of growing-season processes. Because sulfur flows are highly dependent upon biomass (carbon) flows, key flows of carbon are presented for comparison (Appendix Table 23.1).

The largest single accumulated flows were the uptake and mineralization of sulfate by microbes: about 5.3 g S $\cdot$ m^{-2} followed both routes over the 336-day period and 4.7 g S $\cdot$ m^{-2} over the 182-day period. Although only a small fraction of total sulfate turnover was the result of the action of sulfatase on soil organic esters (3.0% and 3.5% over the two periods), sulfatase release was comparable in magnitude to total root uptake (94% and 77% of root uptake), indicating that this could be a significant source for plant growth.

Total root uptake over the two periods was 0.188 and 0.231 g S $\cdot$ m^{-2}, of which 0.158 and 0.156 g S $\cdot$ m^{-2} was translocated to shoots. Of the quantity translocated, 0.01 g S $\cdot$ m^{-2} (only 6% of total shoot uptake) was remobilized during senescence and retranslocated to roots. As noted, sulfur remobilization was significantly underestimated, perhaps by 2-fold.

For the two periods, microbes assimilated 0.462 and 0.191 g S $\cdot$ m^{-2} of metabolic-litter sulfur, 0.583 and 0.415 g S $\cdot$ m^{-2} of structural-litter sulfur, 0.122 and 0.106 g S $\cdot$ m^{-2} of humad sulfur, and 0.002 and 0.003

TABLE 23.3. ACCUMULATED SULFUR FLOWS, 20 APRIL 1975 TO 21 MARCH 1976 (336 DAYS, g S·m^{-2})

Flows from	Flows to													Total outputs
	Shoots	Standing dead	Roots	Metabolic litter	Structural litter	Sulfate	Reduced inorganic	Grazing nematodes	Microbes	Saprophagic nematodes	Humads	Resistant organic	Atmosphere	
Shoots		0.147	0.01											0.157
Standing dead				0.052	0.069									0.121
Roots	0.158			0.196	0.043	0.004		0.015						0.416
Metabolic litter									0.462		0.431		0.012	0.905
Structural litter									0.583		0.015			0.598
Sulfate			0.188						5.36					5.55
Reduced inorganic						0.250								0.250
Grazing nematodes				0.029	0.013									0.042
Microbes				0.437	0.560	5.34	0.387	0.002/0.028[†]		0.006				6.75
Saprophagic nematodes				0.033	0.0033									0.0066
Humads						0.014			0.122			0.122		0.258
Resistant organic						0.162			0.002					0.164
Atmosphere				0.012										0.012
Total inputs	0.158	0.147	0.198	0.729	0.688	5.77	0.387	0.045	6.53	0.006	0.446	0.122	0.012	15.23 system flux

* Each matrix element indicates the accumulated flow from the row heading to the column heading, thus row sums show total outputs and column sums show total inputs for individual state variables.

[†] fungi/bacteria

TABLE 23.4. ACCUMULATED SULFUR FLOWS, 21 MARCH 1976 TO 20 SEPTEMBER 1976 (182 DAYS, $g\ S \cdot m^{-2}$)

Flows from	Flows to													Total outputs
	Shoots	Standing dead	Roots	Metabolic litter	Structural litter	Sulfate	Reduced inorganic	Grazing nematodes	Microbes	Saprophagic nematodes	Humads	Resistant organic	Atmosphere	
Shoots		0.124	0.009											0.133
Standing dead				0.041	0.034									0.075
Roots	0.156			0.120	0.031	0.001		0.012						0.320
Metabolic litter									0.191		0.238		0.005	0.434
Structural litter									0.415		0.011			0.426
Sulfate			0.231						4.68					4.911
Reduced inorganic						0.224								0.224
Grazing nematodes				0.026	0.011									0.037
Microbes				0.245	0.247	4.67	0.133	0.0015/0.032*		0.003				5.331
Saprophagic nematodes				0.0014	0.0014									0.0028
Humads						0.015			0.106			0.106		0.227
Resistant organic						0.164			0.003					0.167
Atmosphere				0.005										0.005
Total inputs	0.156	0.124	0.240	0.438	0.324	5.074	0.133	0.045	5.395	0.003	0.249	0.106	0.005	12.29 system flux

* fungi/bacteria

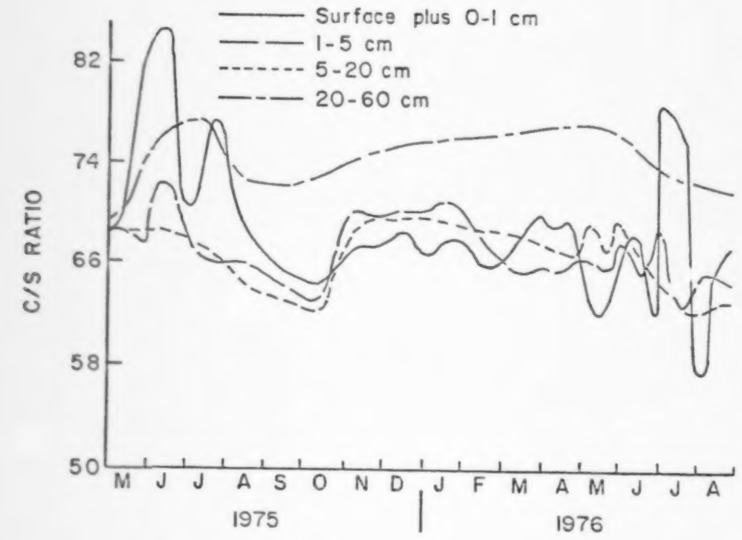


Figure 23.6. C/S ratio of bacteria and actinomycetes.

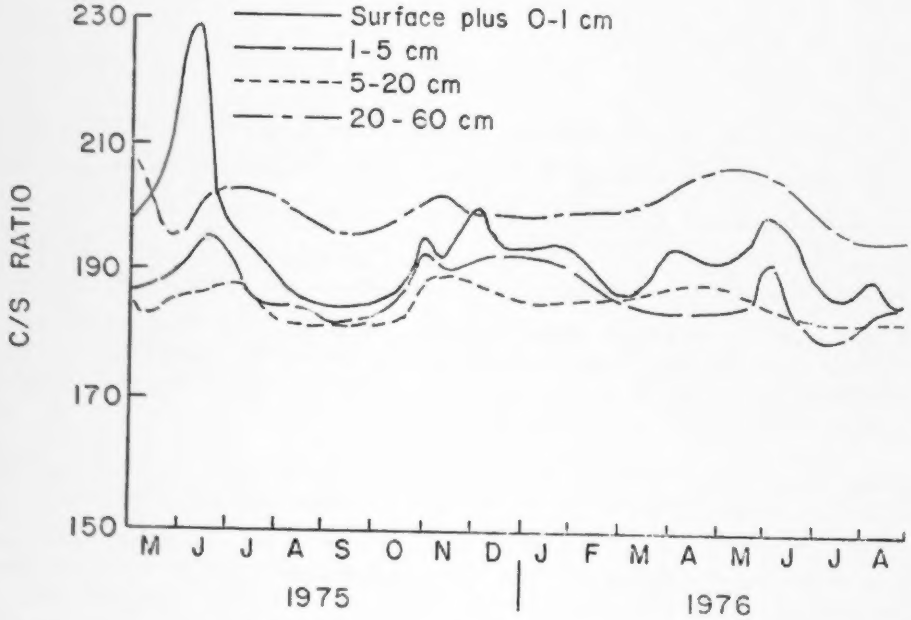


Figure 23.7. C/S ratio of fungi.

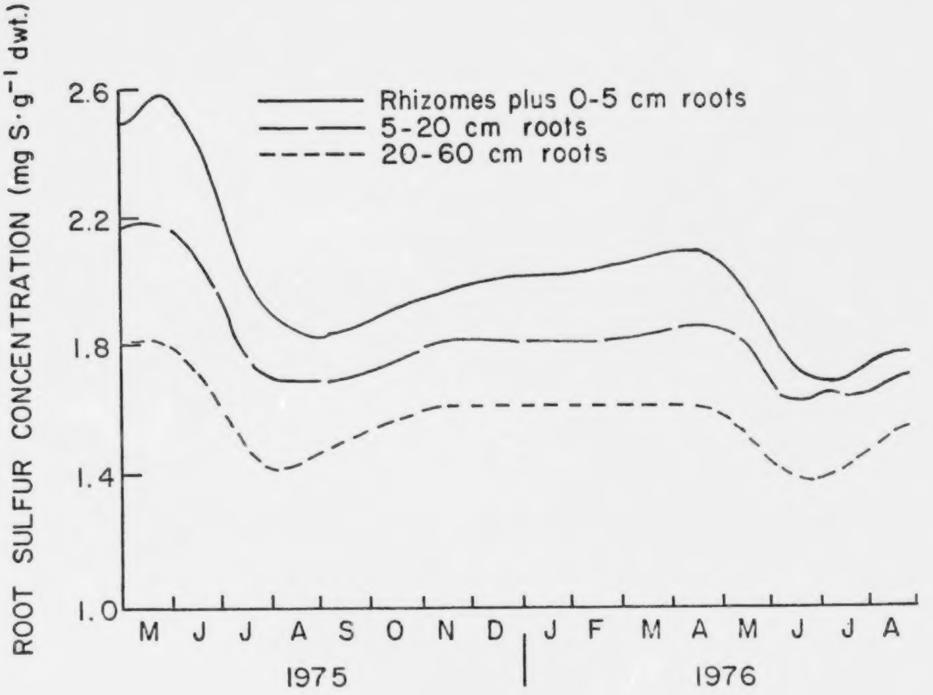


Figure 23.8. Sulfur concentration in *A. smithii* live roots, by three rooting layers.

g S · m^{-2} of resistant-organic-matter sulfur. During decomposition of metabolic litter, only 0.012 and 0.005 g S · m^{-2} flowed to volatile forms. Microbial death returned 0.437 and 0.245 g S · m^{-2} of metabolic litter, and 0.560 and 0.247 g S · m^{-2} of structural litter. The total quantity of sulfur gained by microbes during organic substrate decomposition was 1.169 and 0.715 g S · m^{-2}. Because these amounts were greater than microbial growth demands, net mineralization took place during decomposition. Because net mineralization was of organic substrate sulfur, the question arises: Why are the uptake levels of inorganic sulfur so high?

Grazing nematodes consumed 0.015 and 0.012 g S · m^{-2} of root sulfur, or 8% and 5% of total root uptake. Bacterial consumption by microbivorous nematodes (0.006 and 0.003 g S · m^{-2}) was surpassed by the bacterial consumption of soil larvae forms of the herbaceous nematodes simply because of the difference in their population sizes. These small quantities of accumulated-sulfur flows show that the role of microbivorous nematodes in sulfur cycling is quite small.

Because it was assumed that there were no inputs or outputs to the system as a whole, the sum of inputs to all individual components necessarily equals the sum of outputs to all individual components. This quantity is the total flux of sulfur within the ecosystem: 15.2 g S $\cdot$ m^{-2} over the 336-day period and 12.3 g S $\cdot$ m^{-2} over the 182-day period. There was a total of 96 g S $\cdot$ m^{-2} of sulfur in the model system, of which 78 g S $\cdot$ m^{-2} were in humads and resistant organic material and therefore not involved in rapid recycling. Hence, the turnover rate of the active pool was approximately 15/18, or 0.83 yr^{-1}. The calculated turnover rate of sulfate was 0.36 yr^{-1}; of metabolic litter, 10.0 yr^{-1}; of structural litter, 1.2 yr^{-1}; and of roots, 0.2 yr^{-1}. In comparison, the turnover rate of humad sulfur by decomposition was 0.015 yr^{-1}, the turnover rate of resistant organic sulfur by decomposition was 3×10^{-5} yr^{-1}, and the turnover rate by sulfatase activity of HI sulfur in humads and resistant organic components was 0.004 yr^{-1}.

Net gains or losses to individual components were calculated from the difference between total inputs and outputs (Table 23.5). There were significant net losses from roots, metabolic litter, and microbes. Significant net gains accrued to sulfate, reduced inorganic sulfur, and humads. Because there were no sulfur fluxes across the boundaries of the system, the total reapportionment of sulfur among intra-system components could be calculated as the sum of either all net gains or all net losses. This reapportionment was 0.661 and 0.334 g S $\cdot$ m^{-2} over the 336- and 182-day periods. Because only 4% and 2% of the total system fluxes were reapportioned, flows between state variables were consistent with one another, the whole sulfur cycling system was in equilibrium, and individual system components were maintained near steady-state.

An assumption used in model formulation was that flows of sulfur paralleled flows of biomass during growth, death, and heterotrophic consumption. These flows could be calculated only in conjunction with submodels describing the activities of the pertinent organisms. However, decomposition and mineralization decoupled individual elements, which reduced the dependence of mineral flows on biomass or carbon flows. Because of this decoupling, nutrient cycles must be considered individually. The behavior of each element in its inorganic form is unique; for example, flows into and out of the sulfate pool (such as soil sulfate adsorption, root and microbial uptake, mineralization, sulfatase activity, and gypsum precipitation) are specific to the sulfur cycle. Decoupling also affects the relationship between elements in the organisms; for example, plant C/S or N/S ratios could not be calculated without considering the availability of inorganic sulfur in the soil.

Likewise, C, N, and S mineralizations from organic materials were not formulated as strictly parallel processes. Microbial release and uptake of

TABLE 23.5. NET INCREMENTS OR LOSSES IN INDIVIDUAL COMPARTMENTS (g S · m^{-2}) OVER TWO PERIODS OF SIMULATION. TOTAL REAPPORTIONED SULFUR IN THE SYSTEM IS THE SUM OF ALL NET LOSSES OR GAINS

Compartment	336 days	182 days
Shoots	0.001	0.023
Standing dead	0.026	0.049
Roots	-0.218	-1.08
Metabolic litter	-0.176	0.004
Structural litter	0.09	-0.102
Sulfate	0.22	0.163
Reduced inorganic	0.137	-0.091
Grazing nematodes	0.003	0.008
Microbes	-0.22	0.064
Saprophagic nematodes	-0.0006	0.0002
Humads	0.188	0.022
Resistant organic	-0.042	-0.061
Atmosphere	0.0	0.0
Total reapportioned	~0.6610	~0.3345

each element depends on the relationships among microbial requirements, the nutrient concentrations of the substrates, and the ability of the microbe to draw from the inorganic pool. Thus, nitrogen could be mineralized while sulfur was immobilized. By explicitly considering the sulfur requirements of the microbes in relation to the sulfur gained through sulfate uptake and heterotrophic decomposition, we did not need to use substrate C/S ratios to predict mineralization.

The dynamic framework provided by such a simulation approach should be useful in assessing the impact of additions and losses of sulfur to the system. The model-building process revealed several subjects for further research, particularly on (a) microbial and root sulfate uptake kinetics, (b) sulfatase activity, (c) the origin of soil sulfate esters, (d) the effects of sulfur on plant and microbial metabolism, and (e) maximum microbial mineralization rates.

The model has been invaluable as a tool for integrating data, concepts, and assumptions and, for now, seems to be the best way to deal with the many interacting processes involved in nutrient cycles.

CONCLUSIONS

The simulation model described here proved to be a valuable conceptual and experimental tool. Much was learned while constructing the model and is currently being applied to second generation sulfur deposition and cycling models.

REFERENCES

Alexander, M. 1971. Introduction to Soil Microbiology. John Wiley and Sons, Inc., New York. 472 pp.

Attoe, V. J., and R. A. Olson. 1966. Factors Affecting Rate of Oxidation in Soils of Elemental Sulfur and that Added in Rock Phosphate-sulfur Fusions. Soil Sci., 101:317-325.

Banwart, W. L., and J. M. Bremner. 1976. Evolution of Volatile Sulfur Compounds from Soils Treated with Sulfur-containing Organic Materials. Soil Biol. Biochem., 8:439-443.

Barrien, B. S., and J. G. Wood. 1939. Studies on the Sulfur Metabolism of Plants. II. The Effect of Nitrogen Supply on the Amounts of Protein Sulphur and on the Value of the Ratio of Protein Nitrogen to Protein Sulphur in Leaves at Different Stages During the Life Cycle of the Plant. New Phytol., 38:257-264.

Barrow, N. J. 1960a. The Effects of Varying the Nitrogen, Sulphur, and Phosphorus Content of Organic Matter on its Decomposition. Aust. J. Agric. Res., 11:317-330.

Barrow, N. J. 1960b. A Comparison of the Mineralization of Nitrogen and of Sulphur from Decomposing Organic Materials. Aust. J. Agric. Res., 11:961-969.

Barrow, N. J. 1961. Mineralization of Nitrogen and Sulphur from Sheep Feces. Aust. J. Agric. Res., 11:645-650.

Barrow, N. J. 1967. Studies on the Adsorption of Sulfate by Soils. Soil Sci., 104:342-349.

Bell, G. K. 1975. Types of Sulphur Fertilizer Made in Australia and Their Place in Agriculture. In: K. D. McLachlan, ed. Sulphur in Australian Agriculture. Sydney University Press, Sidney. 261 pp.

Bettany, J. R., J. W. B. Stewart, and E. H. Halstead. 1973. Sulfur Fractions and Carbon, Nitrogen, and Sulfur Relationships in Grassland, Forest, and Associated Transitional Soils. Soil Sci. Soc. Am. Proc., 37:915-918.

Bettany, J. R., J. W. B. Stewart, and E. H. Halstead. 1974. Assessment of Available Soil Sulphur in an ^{35}S Growth Chamber Experiment. Can. J. Soil Sci., 54:309-315.

Biddulph, O., S. Biddulph, R. Cory, and H. Koontz. 1958. Circulation Patterns for Phosphorus, Sulfur and Calcium in the Bean Plant. Plant Physiol., 33:293-300.

Chaudry, I. A., and A. H. Cornfield. 1967. Effect of Moisture Content During Incubation of Soil Treated with Organic Materials on Changes in Sulphate and Sulphide Levels. J. Sci. Fd. Agric., 18:38-40.

Chao, T. T., M. E. Harward, and S. C. Fang. 1962. Adsorption and Desorption Phenomena of Sulfate Ions in Soils. Soil Sci. Soc. Am. Proc., 26:234-237.

Clark, F. 1977. Internal Cycling of ^{15}Nitrogen in Shortgrass Prairie. Ecology, 58:1322-1333.

Cole, C. V., G. S. Innis, and J. W. B. Stewart. 1977. Simulation of Phosphorus Cycling in Semiarid Grasslands. Ecology, 58:1-15.

Cooper, P. J. M. 1972. Aryl Sulphatase Activity in Northern Nigerian Soils. Soil Biol. Biochem., 4:333-337.

Coughenour, M. B. 1978. Grassland Sulfur Cycle and Ecosystem Responses to Low-level SO_2. Ph.D. Diss. Colorado State Univ., Fort Collins, Colorado. 353 pp.

Detling, J. K., W. J. Parton, and H. W. Hunt. 1979. A Simulation Model of *Bouteloua gracilis* Biomass Dynamics on the North American Shortgrass Prairie. Oecologia (Berl.), 38:167-191.

Dodd, J. L., W. K. Lauenroth, G. L. Thor, and M. B. Coughenour. 1978. Effects of Chronic Low Level SO_2 Exposure on Producers and Litter Dynamics. In: E. M. Preston and T. L. Gullett, eds., Bioenvironmental Impact of a Coal-fired Power Plant, Fourth Interim Report, Colstrip, Montana. EPA-600/3-79-044. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 384-493.

Dutt, G. R. 1962. Prediction of the Concentration of Solutes in Soil Solutions for Soil Systems Containing Gypsum and Exchangeable Ca and Mg. Soil Sci. Soc. Am. Proc., 26:341-343.

Dutt, G. R. 1964. Effects of Small Amounts of Gypsum in Soils on the Solutes in Effluents. Soil Sci. Soc. Am. Proc., 28:754-757.

Edwards, J. H., and S. A. Barber. 1976. Nitrogen Flux into Corn Roots as Influenced by Shoot Requirement. Agron. J., 68:471-473.

Evans, C., and C. O. Rost. 1945. Total Organic Sulfur and Humus Sulfur of Minnesota Soils. Soil Sci., 59:125-137.

Ferris, H. 1976. Development of a Computer-simulation Model for a Plant-nematode System. J. Nematol., 8:255-263.

Frederick, L. R., R. L. Starkey, and W. Segal. 1957. Decomposability of some Organic Sulfur Compounds in Soil. Soil Sci. Soc. Am. Proc., 21:287-292.

Freney, J. R. 1960. The Oxidation of Cysteine to Sulphate in Soil. Aust. J. Agric. Res., 11:387-392.

Freney, J. R. 1961. Some Observations on the Nature of Organic Sulphur Compounds in Soils. Aust. J. Agric. Res., 12:424-432.

Freney, J. R. 1967. Sulfur-containing Organics, In: A. P. McLaren and C. H. Peterson, eds. Soil Biochemistry. Vol. 1. Marcel Dekker, Inc., New York. pp. 229-259.

Freney, J. R., and K. Spencer. 1960. Soil Sulphate Changes in the Presence and Absence of Growing Plants. Aust. J. Agric. Res., 11:339-345.

Freney, J. R., and F. S. Stevenson. 1966. Organic Sulfur Transformations in Soils. Soil Sci., 100:307-316.

Freney, J. R., G. E. Melville, and C. H. Williams. 1971. Organic Sulphur Fractions Labelled by Addition of ^{35}S-sulphate to Soil. Soil Biol. Biochem., 3:133-141.

Freney, J. R., F. S. Stevenson, and A. H. Beavers. 1972. Sulfur-containing Amino Acids in Soil Hydrolysates. Soil Sci., 114:468-476.

Frissel, M. J., and P. Reiniger. 1974. Simulation of Accumulation and Leaching in Soils. Centre for Agricultural Publ. and Documentation, Wageningen, Netherlands. 116 pp.

Gustafson, J. D. 1977. SIMCOMP 4.0 Reference Manual. Natural Resource Ecology Laboratory, Fort Collins, Colorado.

Gustafson, J. D., and G. S. Innis. 1973. SIMCOMP Version 3.0 User's Manual. US/IBP Grassland Biome Tech. Rep. No. 218. Colorado State Univ., Fort Collins. 149 pp.

Harward, M. E., T. T. Chao, S. C. Fang. 1962. Soil Properties and Constituents in Relation to Mechanisms of Sulphate Adsorption, In: Radio Isotopes in Soil-plant Nutrition Studies. Int. Atomic Energy Agency, Vienna. pp. 93-114.

Heitschmidt, R. K., W. K. Lauenroth, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulphur Dioxide on Western Wheatgrass in a Southeastern Montana Grassland. J. Appl. Ecol. (submitted).

Houghton, C., and F. A. Rose. 1976. Liberation of Sulfate from Sulfate Esters by Soils. Appl. Environ. Microb., 31:969-976.

Hunt, H. W. 1977. A Simulation Model for Decomposition in Grasslands. Ecology, 58:469-484.

Ingelsten, B. 1966. Absorption and Transport of Sulfate by Wheat at Varying Mannitol Concentration in the Medium. Physiol. Plant, 19:563-579.

Kellog, W. W., R. D. Cadle, E. R. Allen, A. L. Lazrus, and E. A. Martell. 1972. The Sulfur Cycle. Science, 175:587-596.

Kowalenko, C. G., and L. E. Lowe. 1975b. Mineralization of Sulfur from Four Soils and its Relationship to Soil Carbon, Nitrogen and Phosphorous. Can. J. Soil Sci., 55:9-14.

Leggett, J. E., and E. Epstein. 1956. Kinetics of Sulfate Absorption by Barley Roots. Plant Physiol., 31:222-226.

May, P. F., A. R. Till, and A. M. Downes. 1968. Nutrient Cycling in Grazed Pastures, I. A Preliminary Investigation of the Used of [^{35}S] gypsum. Aust. J. Agric. Res., 19:531-543.

May, P. F., A. R. Till, and M. J. Cumming. 1972. Systems Analysis of ^{35}Sulphur Kinetics in Pastures Grazed by Sheep. J. Appl. Ecol., 9:25-49.

McGill, W. B., H. W. Hunt, R. G. Woodmansee, and J. O. Reuss. 1978. Dynamics of C and N in Grassland Soils. Chapter 4. In: J. E. Ellis and W. J. Parton, eds. Impact of Strip-mine Reclamation Practices A Simulation Study. A report to the Western Energy and Land Use Team, Office of Biological Services, U.S. Fish and Wildlife Service.

Metson, A. S. 1973. Sulphur in Forage Crops; Plant Analysis as a Guide to the Sulphur Status of Forage Grasses and Legumes. Tech. Bull. No. 20, The Sulphur Institute, Washington, D.C. 24 pp.

Monheimer, R. H. 1975. Effects of Three Environmental Variables on Sulfate Uptake by Aerobic Bacteria. Appl. Microb., 30:975-981.

Nelson, L. E. 1964. Status and Transformation of Sulfur in Mississippi Soils. Soil Sci., 97:300-306.

Nissen, P. 1971. Uptake of Sulfate by Roots and Leaf Slices of Barley: Mediated by Single, Multiphasic Mechanisms. Physiol. Plant, 24:315-324.

Parton, W. J. 1978. Abiotic Submodel. Chapter 2. In: J. E. Ellis and W. J. Parton, eds. Impact of Stripmine Reclamation Practices: A Simulation Study. A Report to the Western Energy and Land Use Team, Office of Biological Services, U.S. Fish and Wildlife Service.

Parton, W. J., D. M. Swift, and J. E. Ellis. 1978. Chapter 6. In: J. E. Ellis and W. J. Parton, eds. Impact of Strip-mine Reclamation Practices: A Simulation Study. A report to the Western Energy and Land Use Team, Office of Biological Services, U.S. Fish and Wildlife Service, Fort Collins, Colorado.

Peck, H. D., Jr. 1969. Sulfur Requirements and Metabolism of Microorganisms. In: O. H. Muth and J. E. Oldfield, eds. Symposium on Sulfur in Nutrition. Oregon State Univ., Corvallis.

Peck, H. D. 1975. The Microbial Sulfur Cycle. In: Sulfur in the Environment. Missouri Botanical Gardens, St. Louis. 189 pp.

Persson, L. 1969. Labile-bound Sulfate in Wheat-roots: Localization, Nature and Possible Connection to the Active Absorption Mechanism. Physiol. Plant, 22:959-976.

Petrusewicz, K., and A. Macfadyen. 1970. Productivity of Terrestrial Animals, Principles and Methods. IBP Handbook No. 13, International Biological Program. F. A. Davis Company, Philadelphia. 190 pp.

Pettersson, J. 1966. Active and Passive Components of Sulfate Uptake in Sunflower Plants. Physiol. Plant, 19:459-492.

Reiniger, P., M. J. Frissel, and P. Poelstra. 1971. Simulation of Cation Exchange and Movement in Soils. Proc. Symp. on the Use of Isotopes and Radiation in Research on Soil-plant Relationships Including Applications in Forestry. Vienna. pp. 251-259.

Reuss, J. O., and G. S. Innis. 1977. A Grassland Nitrogen Flow Simulation Model. Ecology, 58(2):379-389.

Rogers, W. P. 1969. Nitrogenous Components and Their Metabolism: Acanthocephala and Nematoda. In: M. Forkin and B. T. Scheer, eds. Chemical Zoology. Vol. II. Academic Press, New York. 687 pp.

Santmeyer, P. H. 1956. Studies on the Metabolism of *Panagrellis redivivus* (Nematoda, Cephalobidae). Proc. Helminthol. Soc., 23:30-36.

Smolik, J. D. 1974. Nematode Studies at the Cottonwood Site. US/IBP Grassland Biome Tech. Rep. No. 251. Colorado State Univ., Fort Collins. 80 pp.

Smolik, J. D. 1975. Effect of Soil Temperature and Soil Water on Preproduction of Nematode Populations Indigenous to the Cottonwood Site. US/IBP Grassland Biome Tech. Rep. No. 292. Colorado State Univ., Fort Collins. 25 pp.

Starkey, R. L. 1950. Relations of Microorganisms to the Transformations of Sulfur in Soils. Soil Sci., 70:55-65.

Stewart, B. A., L. K. Porter, and F. G. Viets, Jr. 1966a. Effect of Sulfur Content of Straws on Rates of Decomposition and Plant Growth. Soil Sci. Soc. Am. Proc., 30:355-358.

Stewart, B. A., L. K. Porter, and F. G. Viets. 1966b. Sulfur Requirements for Decomposition of Cellulose and Glucose in Soil. Soil Sci. Soc. Am. Proc., 30:453-456.

Stotzky, G., and A. G. Norman. 1961. Factors Limiting Microbial Activities in Soil. II. The Effect of Sulfur. Archiv fur Mikrobiol., 40:370-382.

Tabatabai, M. A., and J. M. Bremner. 1970. Arylsulfatase Activity of Soils. Soil Sci. Soc. Am. Proc., 34:225-229.

Tanji, K. K. 1969. Solubility of Gypsum in Aqueous Electrolytes as Affected by Ion Association and Ionic Strengths up to 0.15 m and at 25°C. Environ. Sci. Tech., 3:656-661.

Till, A. R., and P. F. May. 1970. Nutrient Cycling in Grazed Pastures, II. Further Observations With [^{35}S] Gypsum. Aust. J. Agric. Res., 21:253-260.

Till, A. R., and P. F. May. 1971. Nutrient Cycling in Grazed Pastures, IV. The Fate of Sulphur-35 Following its Application to a Small Area in a Grazed Pasture. Aust. J. Agric. Res., 22:391-400.

Tuovinen, O. H., B. C. Kelley, and D. S. Nicholas. 1975. The Uptake and Assimilation of Sulphate by *Thiobacillus ferroxidans*. Arch. Microbiol., 105:123-127.

Walker, T. W. 1957. The Sulphur Cycle in Grassland Soils. J. Br. Grassl. Soc., 12:10-18.

Williams, R. F. 1955. Redistribution of Mineral Elements During Development. Ann. Rev. Plant Physiol., 6:25-42.

Williams, C. H. 1967. Some Factors Affecting the Mineralization of Organic Sulphur in Soils. Plant Soil, 26:205-223.

Williams, C. H. 1975. The Chemical Nature of Sulphur Compounds in Soils, In: K. D. McLahlan, ed. Sulphur in Australian Agriculture. Sydney Univ. Press, Sydney. 261 pp.

APPENDIX 23.1

Graphical Form of Functions Used in the Model

APPENDIX 23.1 FIGURE TITLES

a. Effect of soil temperature on microbial activity, $E_m(T)$ (taken from McGill et al., 1978).

b. Effect of soil moisture on microbial activity, $E_m(W)$; (a) surface soil layer (b) subsurface soil layers (taken from McGill et al., 1978).

c. Effect of microbial C/S ratio on uptake of solution sulfate, $E_u(CS_m)$ (based on calculated range of microbiol C/S ratios possible).

d. Effect of microbial C/S ratio on mineralization of sulfate, $E_{mn}(CS_m)$ (based on calculated range of microbiol C/S ratios possible).

e. Effect of soil water on proportion of mineralized sulfur which is oxidized (as sulfate) $E_{ox}(W)$ (based on Chaudry and Cornfield, 1967).

f. Effect of soil temperature on sulfatase activity, $E_e(T)$; dashed lines indicate the temperature at which the unmodified rate was determined experimentally (based on Tabatabai and Bremner, 1970).

g. Effect of microbial C/S ratio on decomposition of structural litter (hypothesized).

h. Effect of soil moisture on the proportion of decomposing metabolic litter sulfur which is volatilized, $P_v(W)$ (based on Banwart and Bremner, 1976).

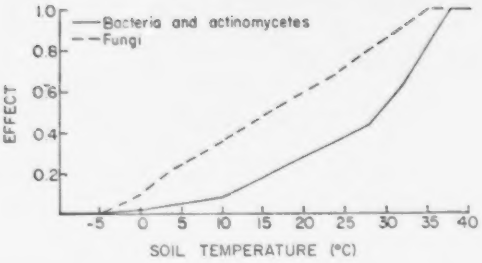
i. Effect of soil moisture on root uptake of sulfate, $E_r(W)$ (modified from McGill et al., 1978).

j. Effect of temperature (°) on root uptake of sulfate, $E_r(T)$ (modified from McGill et al., 1978).

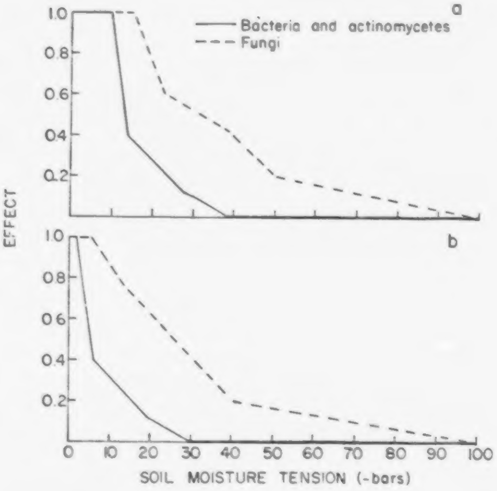
k. Effect of whole plant C/S ratio on root uptake of sulfate, $E_u(CS_P)$ (hypothesized).

l. The ratio of shoot sulfur concentration to live root sulfur concentration as a function of phenology, I(Ph). Plant phenophases are: (1) early vegetative growth (2) middle vegetative growth (3) full vegetative growth (4) reproductive growth (5) ripe fruit (6) senescence (hypothesized).

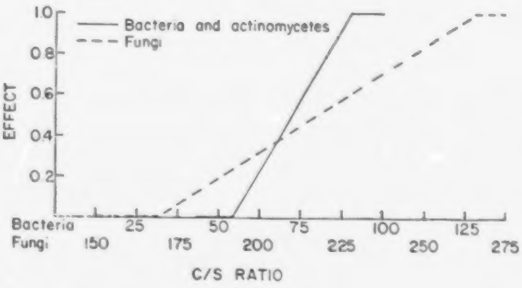
m. Temperature dependent metabolic rate of nematodes, $M_r(T)$ (adapted from Santmeyer, 1956).



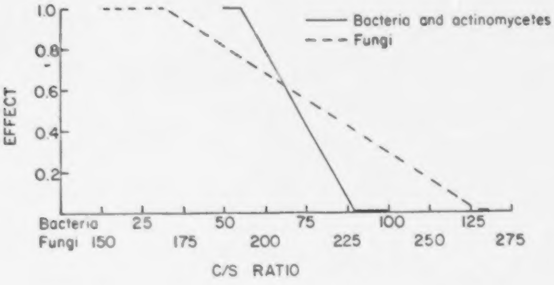
Appendix Figure 23.1-a



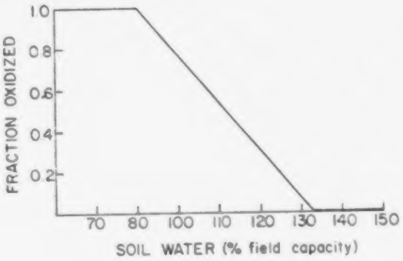
Appendix Figure 23.1-b



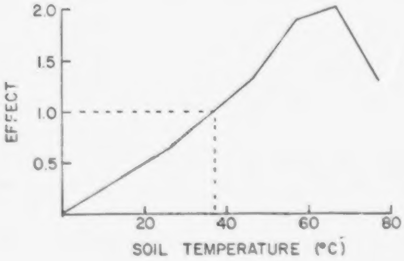
Appendix Figure 23.1-c



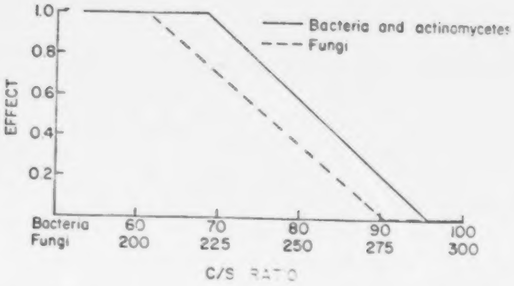
Appendix Figure 23.1-d



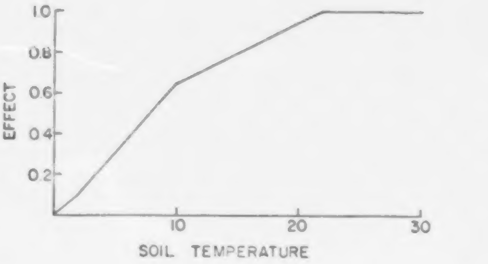
Appendix Figure 23.1-e



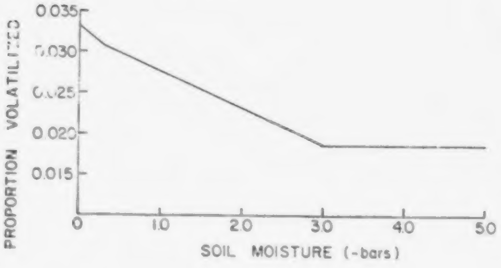
Appendix Figure 23.1-f



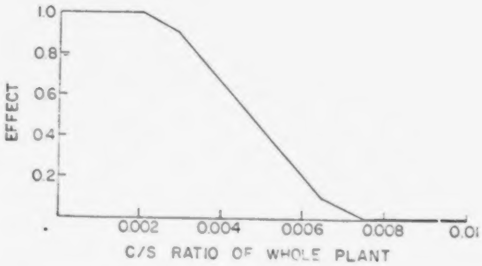
Appendix Figure 23.1-g



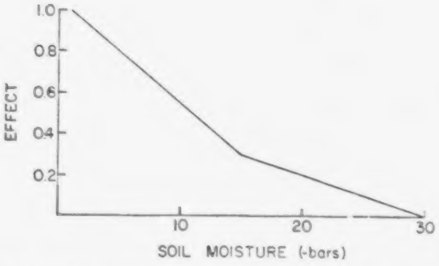
Appendix Figure 23.1-j



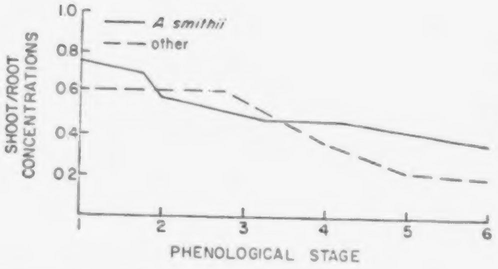
Appendix Figure 23.1-h



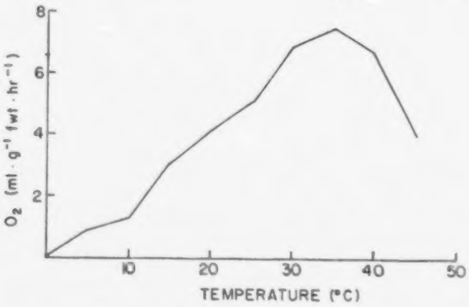
Appendix Figure 23.1-k



Appendix Figure 23.1-i



Appendix Figure 23.1-l



Appendix Figure 23.1-m

APPENDIX 23.2

Comparison Between Observed and Simulated Values for Non-Sulfur Ecosystem Variables

APPENDIX 23.2 FIGURE TITLES

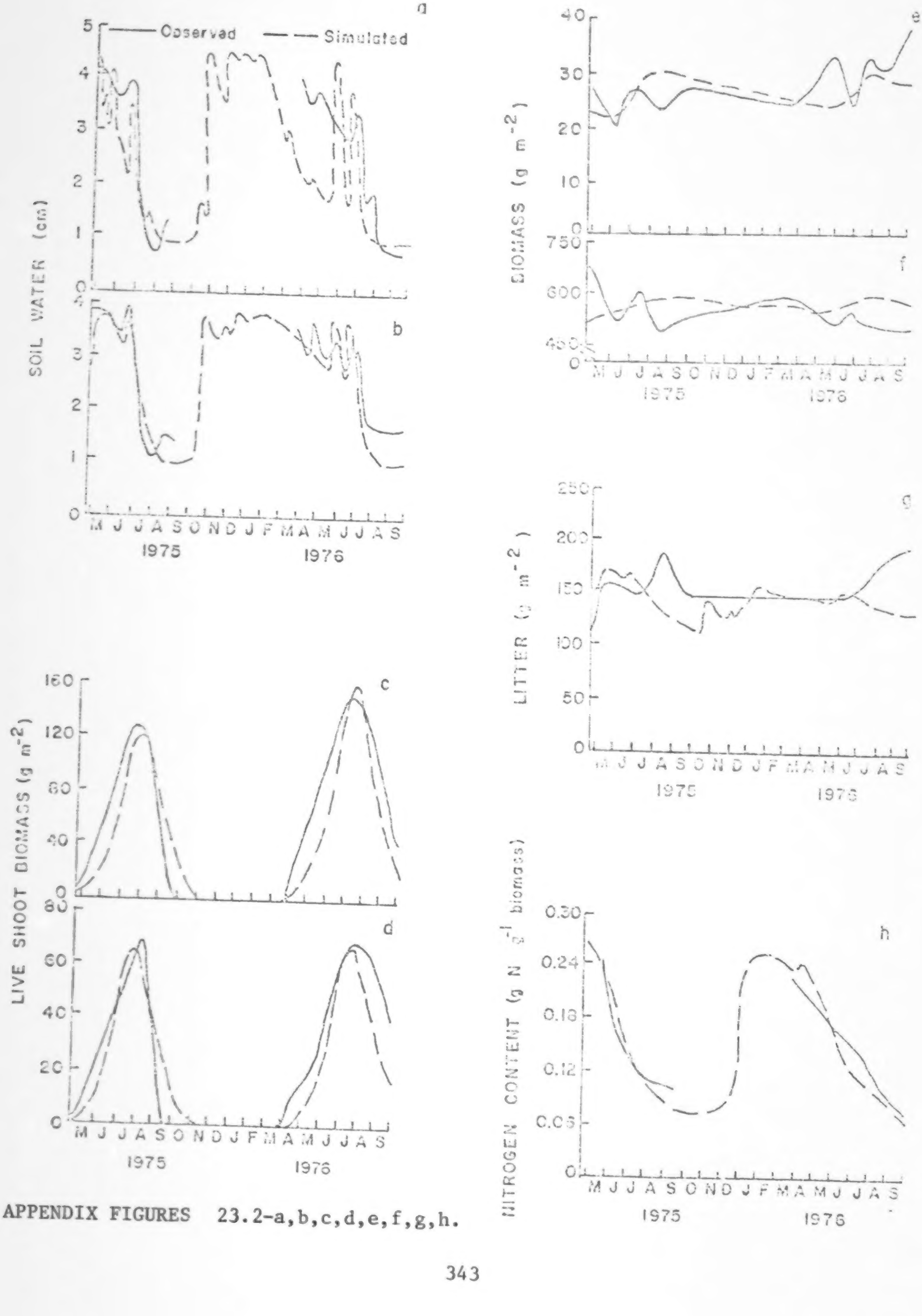
a and b. Observed vs. simulated 0-15 cm soil water (a) and 15-30 cm soil water (b).

c and d. Observed vs. simulated total live shoot biomass (c) and for *Agropyron smithii* live shoot biomass (d).

e and f. Observed vs. simulated rhizome biomass (e) and total root biomass (f).

g. Observed vs. simulated litter biomass.

h. Observed vs. simulated nitrogen content of *Agropyron smithii* live shoots.



APPENDIX FIGURES 23.2-a,b,c,d,e,f,g,h.

APPENDIX TABLE 23.1. ACCUMULATED CARBON FLOWS (g C · m^{-2})

	20 April 1975 to 21 March 1976 (336 days)		21 March 1976 to 20 September 1976 (182 days)		Total (518 days)	
	A. smithii	Other	*A. smithii*	Other	*A. smithii*	Other
A. Primary production						
P_N shoot	90.13	84.41	80.23	112.38	170.36	196.79
P_N plant (NPP)	61.23	55.45	54.90	86.09	116.13	141.54
Shoot growth	30.21	25.76	31.64	45.37	61.85	71.13
Rhizome or crown growth	3.17	8.05	2.21	11.52	6.19	19.57
Root growth	27.85	21.64	21.05	29.20	48.90	50.84
Crown respiration	2.61	7.51	2.29	6.44	4.09	13.95
Root respiration	25.80	20.91	22.78	19.64	48.58	40.55
B. Belowground						
1. Decomposition						
Structural	232.54		176.86		409.04	
Metabolic	18.26		9.45		27.71	
Humad	14.88		12.88		27.76	
Resistant organic	3.37		2.78		6.15	
2. Respiration						
Microbial	118.34		91.29		209.63	
Nematode	1.95		1.76		3.72	
3. Nematodes						
Root consumption	3.43		3.09		6.52	
Fungi consumption	0.358		0.316		0.674	
Bacterial consumption (herbivore soil larvae)	2.02		2.25		4.27	
Bacterial consumption (saprophages)	0.448		0.199		0.647	

SECTION 24

RELATIONSHIP OF SO_2 DEPOSITION TO A GRASSLAND SULFUR CYCLE

M. B. Coughenour

ABSTRACT

A simulation model representing deposition of SO_2 to a grassland ecosystem was constructed. This model was executed simultaneously with other models describing sulfur cycling, primary production, decomposition, and abiotic processes. The model provided fair representation of the accumulation of sulfur on live and dead leaves and soil. A deposition velocity of $V_g = 0.09$ cm $\cdot$ sec^{-1} was calculated from the verified simulation results. As a result of continuous fumigation at 10 pphm SO_2, flows of sulfur through all system components increased by approximately 1.21 g S $\cdot$ m^{-2} $\cdot$ yr^{-1}. Many flows of sulfur in the system increased, especially outflows from live and dead shoots. Total reapportionment of sulfur among system components was 3.8 times greater under the SO_2 exposure, indicating the degree of departure of the system from equilibrium.

INTRODUCTION

Effects of sulfur dioxide pollution have traditionally been limited to assessment of toxic effects on metabolism at the organism level. However, more subtle changes in the functioning of ecosystems may arise from an interaction of the pollutant with the nutrient cycle of which it is a part.

Analyses of the global sulfur budget reveal a major deviation from the pre-industrial state. Man now contributes about half as much sulfur to the atmosphere as nature (Kellogg *et al.* 1972). Globally, 20 x 10^6 metric tons of sulfur (as S) are dry deposited on land (Almqvist 1974). Dry deposition over land areas is probably about one-fourth of that brought down in precipitation, while anthropogenic sources are wet and dry deposited on land at a rate of 24 x 10^6 tons $\cdot$ yr^{-1} each (Granat *et al.* 1976). Moss (1976) estimated that 100 x 10^6 tons are absorbed (as SO_2) by land surfaces, 75 x 10^6 tons are absorbed by vegetation, and 25 x 10^6 tons by soil. Soil absorption downwind from a power plant has been estimated at 49 to 98 kg S $\cdot$ ha^{-1} (Nyborg *et al.* 1973).

These imbalances in the global sulfur cycle must consequently carry over to terrestrial ecosystems, which remove portions of the atmospheric load. The significance of an imbalanced terrestrial sulfur cycle is unknown. The stoichiometry of life itself results in a certain relationship between macronutrients in the biosphere (e.g., H_{2900} O_{1480} C_{1480} N_{16} $P_{1.8}$ S (Payrissat, 1974). It is also conceivable that there exists a homeostatic control over the entire planetary environment, including the chemical compositon of the atmosphere, by and for the biosphere (Lovelock and Margulis 1974). Such a homeostasis would be analogous to the homeostasis of biota with the physical environment that arises during ecosystem development in which there is a closing, or tightening, of nutrient cycles (Odum 1969). Ecosystem homeostasis may be achieved by diversion of excess nutrients into unavailable pools or reduction of losses from such pools so that depleted nutrients may later be restored (Hutchinson 1948).

These hypotheses imply that the specific form of a nutrient cycle is related to the structure and function of the ecosystem. Thus, in assessing the total environmental consequences of SO_2 pollution, all environmental components must be drawn together and links between them should be made explicit. Reference to the sulfur cycle and the tracing of paths excess sulfur must follow would facilitate such an approach (Moss 1976).

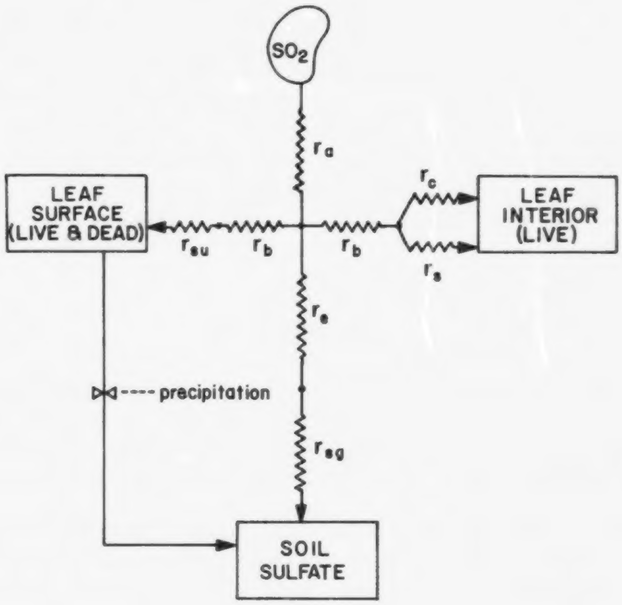


Figure 24.1. Schematic of flows of SO_2-sulfur to various sinks. Resistances to SO_2 transfer are atmospheric boundary layer (ra), leaf boundary layer (rb), leaf cuticular (rc), stomatal (rs), leaf surface (rsu), sub-canopy atmospheric (re), and ground surface (rsg.)

MATERIALS AND METHODS

I developed a submodel (Figure 24.1) that could simulate the SO_2-deposition process and interface with a sulfur-cycling submodel (Figure 24.2) described elsewhere in greater detail (Coughenour *et al.*, 1979). This model relies extensively on established micrometerological relationships presented in the literature. Consequently, this paper does not document the derivations of equations, and the reader is encouraged to examine relevant citations for development and analysis.

To calculate the aerodynamic diffusion resistances within and above the plant canopy, the windspeed profile must first be evaluated. An empirical relation is used to calculate a zero plane displacement

$$d = 0.63 \cdot h \tag{1}$$

where h is the height (cm) of the plant canopy, and roughness length (z_o) follows as

$$z_o \ k(h - d) \tag{2}$$

where k is Von Karman's constant (0.41) (Monteith 1973).

Because the windspeed profile is affected by atmospheric stability, a mean Richardson number is introduced

$$\bar{R}_i = \frac{g\ z\ (T - T_o)}{U_r^2 T} \tag{3}$$

where g is the gravitational constant ($cm^2 \cdot sec^{-1}$), T is reference height temperature (^oK), T_o is the temperature of the surface losing heat (canopy temperature), U_r is reference height windspeed ($cm \cdot sec^{-1}$), and z is height (Monteith 1963, Whelpdale and Shaw 1974).

Friction velocity (U_*) is then estimated with the stability-corrected logarithmic windspeed profile relationship

$$\frac{U_r}{U_*} = \frac{1}{K} \left[\ln \left(\frac{z_r - d}{z_o}\right)\right] (1 - \sigma \bar{R}_i)^{-\frac{1}{2}} \tag{4}$$

where z_r is reference height (cm), σ is an empirical constant (= 10, Monteith 1963), and U_r is reference height windspeed. The windspeed at canopy height h is then

$$U_h = \frac{U_*}{k} \ln \left(\frac{h-d}{z_o}\right) (1 - \sigma \bar{R}_i)^{-\frac{1}{2}} \tag{5}$$

The air layer within and above a canopy can be decomposed into three regions (Inoue, 1963):

1. The lowest part of the atmospheric surface layer (atmospheric boundary layer), usually represented by the logarithmic wind profile.

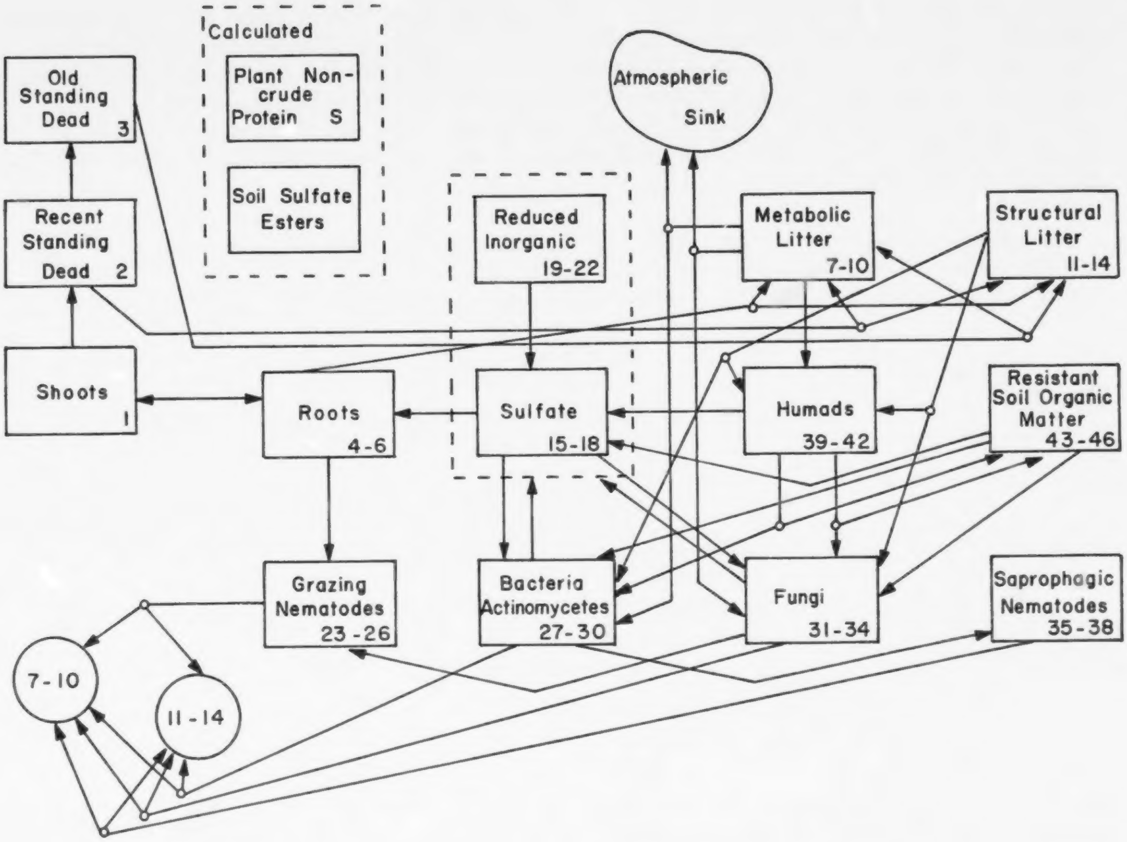

Calculated
Plant Non-crude Protein S
Soil Sulfate Esters
Old Standing Dead 3
Recent Standing Dead 2
Shoots 1
Atmospheric Sink
Reduced Inorganic 19-22
Metabolic Litter 7-10
Structural Litter 11-14
Roots 4-6
Sulfate 15-18
Humads 39-42
Resistant Soil Organic Matter 43-46
Grazing Nematodes 23-26
Bacteria Actinomycetes 27-30
Fungi 31-34
Saprophagic Nematodes 35-38
7-10
11-14

Figure 24.2. Flow diagram showing structure of sulfur cycle model. Large circles with numbers are duplicate representations of metabolic and structural litter. Small circles represent the partitioning of a flow. Flows to the atmospheric sink (volatilization) are assumed to return immediately to metabolic litter. Litter components occur at the soil surface and belowground. Metabolic litter is labile, with variable sulfur composition, while structural litter is more resistant, with invariable sulfur composition. Reduced inorganic refers principally to the sulfide form. Grazing nematodes consume roots and fungi in the adult stage, bacteria in larval stages; saprophagic forms consume only bacteria. Humads are relatively labile and represent soil organic matter adsorbed and complexed with clay minerals. Resistant organic is the highly recalcitrant fraction of soil organic matter. Flows between atmosphere and metabolic litter represent flows of organic volatile sulfur compounds rather than SO_2.

2. The canopy eddy layer, separated from layer 1 at canopy height h, in which the wind profile is more complex.

3. The lowest part of the plant air layer, in which both plants and ground surface influence the profile.

The necessary characteristics of region 1 have been calculated by Equations (1-5). The transition from region 1 to 2 may not be sharp, giving rise to a transitonal region called the Honami-air layer. From this region down into the canopy eddy layer (region 2) the logarithmic profile is replaced by an exponential profile of eddy speeds. The depth of the canopy eddy is

$$lw = 0.473(h - d) \tag{6}$$

and extends down from approximately h (Inoue, 1963). An estimate of the center of the canopy layer is

$$z_c = h - \frac{lw}{2} \tag{7}$$

Within this layer, friction velocity varies with height; therefore, at z_c

$$U_*(z_c) = U_* \, e^{-\alpha(1 - \frac{z_c}{h})} \tag{8}$$

and, similarly, eddy velocity at z_c is

$$U(z_c) = U_h \, e^{-\alpha(1 - \frac{z_c}{h})} \tag{9}$$

where α is defined by the relationship

$$lw = \frac{k}{\alpha} \cdot \frac{h}{\ln(\frac{h-d}{z_o})} \tag{10}$$

(Inoue, 1963).

Over the logarithmic profile of region 1, the resistance to diffusion is

$$r_a = \frac{U_r}{U_*^2} \tag{11}$$

where r_a is aerodynamic resistance (Monteith, 1973). Diffusion through regions 2 and 3 is more complex. A logarithmic profile is resumed in region 3 and the atmospheric resistance from z_c to zero ground displacement may be approximated by

$$r_e = \frac{U(z_c)}{U_*(z_c)^2} \tag{12}$$

An additional atmospheric diffusion resistance is due to a turbulent boundary layer surrounding individual leaves. An empirical correlation has been discovered that incorporates the effect of leaf shape on this resistance as

$$r_b = 1.3(\frac{l}{U_c})^{\frac{1}{2}} \tag{13}$$

(Monteith, 1965), where l is the characteristic length of the leaf and U_c is canopy windspeed. However, r_b varies with $D^{-2/3}$ (D is the diffusivity of the gas). This leads to the relationship

$$r_b = 0.12(\frac{l}{U_c})^{\frac{1}{2}} D^{-2/3} \tag{14}$$

(Kabel *et al.*, 1976), which is the one used in this model. The characteristic length (l) is

$$l = \frac{4A}{P} \tag{15}$$

where A is planar area (cm^2) of the leaf and P the perimeter (cm) (Kabel *et al.*, 1976).

The final step of diffusion into the interior of the leaf requires evaluating diffusive resistance through stomatal pores. Stomatal opening depends upon light, plant water status, and atmospheric CO_2 concentration (Meidner and Mansfield, 1968). Plant water potential was calculated empirically in equations representing responses of *Agropyron dasystachyum* to light and soil moisture (Redman, 1973)

$$\Psi_P = -(10.59 + \frac{538}{m}), \text{ if } G \leq 40 \tag{16}$$

$$\Psi_P = -(0.74 + \frac{497}{m}), \text{ if } G > 40 \tag{17}$$

$$\Psi P = - [4.7 + 0.14(\frac{G}{60})], \text{ if } m > 24 \tag{18}$$

where Ψ_P is plant water potential (bars), m is soil moisture level (% volume), and G is global radiation (ly/min). Stomatal resistance is subsequently a function of Ψ_P and G

$$r_s = \frac{1.2}{G^{(0.2-0.2\Psi_P)}}, \text{ if } \Psi_P > -15 \tag{19}$$

$$r_s = \frac{1.2\ e^{-1.4(\Psi_P+15.0)}}{G^{(0.2-0.02\Psi_P)}}, \text{ if } \Psi_P \leq -15 \tag{20}$$

which are empirical relationships derived from data for *Agropyron dasystachyum* and *A. smithii* (Ripley and Saugier, 1975). Since this resistance is applicable to water vapor diffusion, the value is converted by

$$r_{SO_2} = r_{H_2O} \frac{D_{H_2O}}{D_{SO_2}} \tag{21}$$

where r is the resistance and D the diffusivity of the subscripted gas species.

An alternate pathway into the leaf interior is through the cuticle itself. Although this resistance is large, it is incorporated here for completeness. Cuticular resistance (r_c) will be assumed at 80 sec · cm^{-1}. When resistances are in parallel as for r_s and r_c, the total resistance over the circuit is

$$r = \frac{r_s \cdot r_c}{r_s + r_c} \tag{22}$$

Resistance in series such as r_a and r_b combine linearly as

$$r = r_a + r_b \tag{23}$$

The total resistance of the path into the leaf may then be written

$$r = r_a + r_b + \frac{r_s \cdot r_c}{r_s + r_c} \tag{24}$$

One further resistance (r_m) may be postulated that expresses the capacity of the mesophyllic cell surfaces to remove the gas for the substomatal cavity. SO_2 is very highly soluble in water and is probably rapidly oxidized to SO_3= on the mesophyllic surface. Thus, r_m may be negligible when the plants are able to remove SO_3= from the mesophyll solution at a greater rate than it is formed. Only when rate of entry exceeds this removal rate might r_m become significant (Unsworth *et al.* 1976, Bennet *et al.* 1973). However, no measurements of r_m are available (Unsworth *et al.* 1976). Since r_b and r_s are known, r_m must be adjusted to match observed values for accumulation of sulfur on live leaves (after r_{su} is found).

Passive deposition onto leaf surfaces is governed by the sink strength of the surface. This sink strength is represented here as a constant leaf surface resistance (r_{su}). The value of r_{su} was found by trial and error to most closely match observed and predicted values of sulfur accumulation on dead leaves. The total resistance for deposition onto live or dead leaf surfaces is then

$$r = r_a + r_b + r_{su} \tag{25}$$

In this model, flux to the soil is found in a similar fashion by use of

$$r = r_a + r_e + r_{sg} \tag{26}$$

where r_a and r_e are the atmospheric resistances to diffusion above and below the canopy, and r_{sg} expresses the surface resistance of the ground. Estimates of soil surface resistance range from 0.24 sec · cm^{-1} (Payrissat and Beilke, 1975) to 5 sec · cm^{-1} (Seim, 1970). This suggests that no single value is applicable in all circumstances. A surface resistance of 20 sec · cm^{-1} was necessary to approximate the observed values of sulfate accumulation in the surface soil layer.

The resistances of diffusion to each of the various sinks calculated above are finally implemented to yield an absolute flux occurring over the solution interval of the simulation (one day). The relationship

$$F = \frac{C}{r} \tag{27}$$

is used (Garland *et al.*, 1973; Shepard, 1974) to facilitate this calculation, where F is flux rate (μg SO_2 · cm^{-2} · sec^{-1}), C is concentration (μg SO_2 · cm^{-3}) and r is total resistance from the point at which C was measured to the sink (sec · cm^{-1}). When C is measured at z_r, r_a must be included in r, but when C is measured in the canopy (as is the case here) r_a need not be included in r. Flux to leaf surfaces or interiors is

$$F = \frac{C}{r} \cdot \text{LAI} \tag{28}$$

where LAI is leaf area index. Flux through stomates is evaluated two times for each daily period of sunlight as r_s changes in response to diurnal variation in light (G). Fluxes over each of the two time increments are summed to yield daytime flux. This is then added to a nighttime flux, when only the cuticular path is assumed operative.

Inorganic sulfur is leached from leaf surfaces as a function of the intensity and quantity of rain (Tukey, 1966). Recent dead *Agropyron smithii* at the end of the fumigation year in September 1975 averaged about 0.44% sulfur. Over winter this level declined in the absence of fumigation to about 0.11% sulfur in old dead leaves the following March. Over this period there was 16.6 cm precipitation yielding the empirical concentration loss rate of 0.02% sulfur per cm of precipitation used in the model. This is an approximation because a significant portion of the precipitation fell as snow and the intensity of the precipitation was not considered.

RESULTS AND DISCUSSION

Model performance was verified in a simulation that included interactions with sulfur cycling and ecosystem level models (Coughenour *et al.*, 1979). This simulation represented the continuous field fumigation at 10 pphm SO_2 (canopy concentration) beginning on 10 May 1975 and ending 31 October 1975. Verification of the SO_2 deposition submodel was accomplished primarily through comparison between observed and simulated values of live and dead *A. smithii* shoot sulfur concentrations (Figure 24.3). The comparison for live *A. smithii* shoots displayed fairly acceptable agreement, except for the underestimation of deposition in the early part of the season. The overall rate of accumulation, however, seemed correct. The model satisfactorily represented the changes in concentration of sulfur on old dead leaf surfaces except in the latter part of the season, when simulated values rose above observed. The model simulated a 2.5-fold increase in soil sulfate in the top 5-cm soil layer due to SO_2 deposition over two consecutive growing seasons, in agreement with field determinations.

The total flux of SO_2 to the ecosystem amounted to 2.03 g $S \cdot m^{-2}$ for the 174 days of fumigation at 10 pphm SO_2. This deposition rate is transformed into a deposition velocity (V_g) for the period.

$$V_g = \frac{\text{flux rate } (\mu g\ SO_2 \cdot cm^{-2} \cdot sec^{-1})}{\text{concentration } (\mu g\ SO_2 \cdot cm^{-3})} = 0.09\ cm \cdot sec^{-1} \qquad (29)$$

The accumulated flows of sulfur over the simulation period (Table 24 .1) may be compared to those from the nonfumigated simulation over the same period

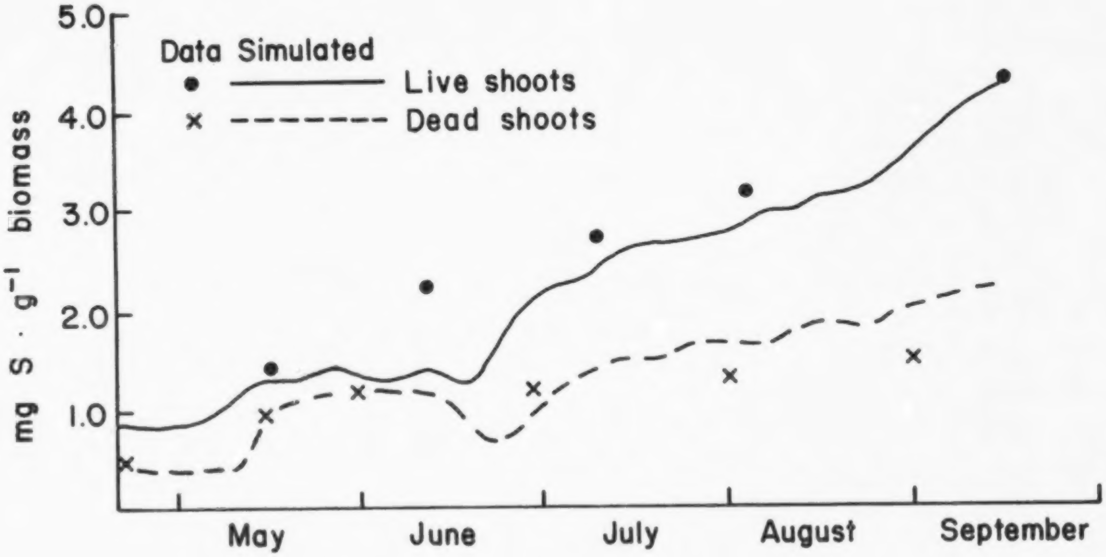


Figure 24.3. Comparison of observed and simulated *Agropyron smithii* shoot sulfur concentrations under 10 pphm SO_2

TABLE 24.1. ACCUMULATED SULFUR FLOWS (g S · m^{-2}) FROM 20 APRIL 1975 TO 21 MARCH 1976 (336 DAYS) UNDER 10 pphm SO_2 (CANOPY CONCENTRATION) FUMIGATION

Flows from	Flows to													Total outputs
	Shoots	Standing dead	Roots	Metabolic litter	Structural litter	Sulfate	Reduced inorganic	Grazing nematodes	Microbes	Saprophagic nematodes	Humads	Resistant organic	Atmosphere	
Shoots		0.181 0.481[†]	0.03			0.246								0.938
Standing dead				0.251	0.070	0.377 0.074**								0.772
Roots	0.052			0.205	0.045	0.005		0.015						0.322
Metabolic litter									0.582		0.513		0.016	1.111
Structural litter									0.587		0.0[illegible]			0.602
Sulfate			0.188						5.260					5.448
Reduced inorganic						0.252								0.252
Grazing nematodes				0.030	0.014									0.044
Microbes				0.442	0.579	5.216	0.418	0.002 0.030[‡]		0.006				6.692
Saprophagic nematodes				0.003	0.003									0.007
Humads						0.006			0.046			0.046		0.981
Resistant organic						0.056			0.001					0.057
Atmosphere	0.427 0.484[†]	0.422	0.0225	0.016	*	0.713								2.062
Total inputs	0.963	1.084[§]	0.218	0.947	0.711	6.945	0.423	0.047	6.476	0.006	0.528	0.046	0.016	18.40 system flux

* Total outputs or inputs to each compartment are obtained by summing across the row and down the column respectively.

[†] surface/internal

**leaching/death

[‡] fungi/bacteria

[§] translocation

to assess the impacts of SO_2-sulfur inputs on the sulfur cycle. First, the total system flux (TSF) is defined as

$$TSF = \sum_{i}^{n} \sum_{j}^{n} FLOW_{ij} \tag{30}$$

where $FLOW_{ij}$ is the accumulated flow from state variable i to state variable j, and n is the total number of state variables. The increase in TSF due to SO_2 sulfur inputs for the 336-day simulation period is

$$\Delta TSF = TSF_{SO_2} - TSF_{control} = 3.17 \text{ g S} \cdot m^2 \tag{31}$$

A significant portion of ΔTSF is a direct result of the atmospheric flows. If these flows are subtracted from ΔTSF, the indirect effects of SO_2 on TSF may be found,

$$\Delta TSF_{indirect} = \Delta TSF - \sum_{j}^{m} FLOW_{kj} \tag{32}$$

where k is the index of the atmospheric compartment, and m is the total number of flows from the atmosphere. This indirect effect was 1.12 g S · m^{-2} for the 336-day period.

Perturbations to individual flows are expressed by the ratio of the accumulated flow in the SO_2-fumigated simulation to that in the control simulation (Table .2). The greatest positive perturbations occurred in flows from shoots to standing dead, shoots to roots, standing dead to metabolic litter, and leaching from live and dead shoots to soil sulfate. As a result of greater inflows to metabolic litter, there were also greater outflows from metabolic litter to microbes, humads, and volatile organic forms. As flows from shoots to roots increased, so did flows from roots to litter and sulfate. Because of greater flows from litter and sulfate to microbes, there were greater flows from microbes to litter and nematodes. Finally, increased inputs to nematodes from microbes resulted in greater outflows from nematodes to litter. No perturbations occurred in outflows from structural litter because this component was assumed to have an invariable sulfur composition. Although there was no net change in root sulfate uptake over the whole period, this flow was in fact greater in the fumigated simulation for most of the growing season. Root uptake was, however, accelerated in the control simulation in the period following the growing season. This compensated for the sulfur used in shoot growth. The same phenomena held true for microbial sulfate uptake. Fumigation stimulated microbial uptake during the growing season, but uptake in the control simulation was accelerated thereafter and eventually surpassed that in the fumigated simulation.

Only one flow was consistently suppressed due to fumigation, that from roots to shoots. This was the result of a mechanism in the model that depresses upward translocation as shoot sulfur demands are progressively fulfilled. Uptake from the atmosphere reduced shoot demands. Flows from humads

TABLE 24.2. THE RATIO OF ACCUMULATED SULFUR FLOWS IN THE 10 pphm FUMIGATION SIMULATION (TABLE 24.1) TO SULFUR FLOWS IN THE NON-FUMIGATED CONTROL SIMULATION FOR THE 336 DAY PERIOD

Flows from	Flows to												
	Shoots	Standing dead	Roots	Metabolic litter	Structural litter	Sulfate	Reduced inorganic	Grazing nematodes	Microbes	Saprophagic nematodes	Humads	Resistant organic	Atmosphere
Shoots		4.50	3.00			∞							
Standing dead				4.82	1.01	∞							
Roots	0.33			1.04	1.05	1.30		1.00					
Metabolic litter									1.26		1.19		1.33
Structural litter									1.00		1.00		
Sulfate			1.00						1.04				
Reduced inorganic						1.00							
Grazing nematodes				1.03	1.08								
Microbes				1.01	1.03	0.98	1.08	1.07		1.07			
Saprophagic nematodes				1.06	1.06								
Humads						0.43			0.38			0.38	
Resistant organic						0.34			0.50				
Atmosphere	∞	∞	∞	1.33		∞							

* The ratio is infinite when the denominator is assumed to be zero. Pertubations to flows were progressively damped or they became more removed from the sinks directly removing SO_2 from the atmosphere.

and resistant organic matter did not actually decrease in response to added sulfur but were lower in the fumigated simulation because organic soil sulfur was lower in the fumigated than in the control field plots.

The impact of SO_2 on sulfur cycling is further displayed in the difference between total inputs and outputs to each compartment, which is equivalent to the net change in the compartment (Table 24.3). When inputs and outputs to a compartment are equal, that compartment is at steady state. Soil sulfate was perturbed from steady state by SO_2 inputs more than by any other state variable. The relative SO_2-induced perturbations from steady-state behavior for each compartment were SO_4 > standing dead > humads > roots > reduced organic > resistant organic > shoots > structural litter > metabolic litter > microbes > nematodes.

When either all net gains or all net losses are summed, a total reapportionment among system components is derived (Table 24.3). This may be taken as an aggregate measure of all compartment steady states or of system equilibrium, since if all components were in balance, there would be zero reapportionment. Significantly, this measure was 38 times greater under the continuous SO_2 exposure. This reapportionment principle also would apply to the biosphere and to the "Gaia" (Loveland and Margulis 1974), in that there has been a shift in the equilibrium between sulfur locked away in fossil fuels, sulfur in the atmosphere, and sulfur in terrestrial ecosystems.

CONCLUSIONS

A deposition velocity to the ecosystem as a whole was derived from simulation modeling. The value was approximately .1 cm sec^{-1}. At this rate, with 10 pphm SO_2 maintained continuously, 48 kg S ha^{-1} yr^{-1} could be removed from the atmosphere. At 2 pphm about 10 kg S ha^{-1} yr^{-1} could be removed.

Sulfur absorbed by the ecosystem is a contaminant in the sense that the mean biogeochemical compositions are significantly altered, as are internal recycling rates, and reapportionments. Whether this contamination is harmful or adverse, depends on the relationships of other ecosystem processes to the sulfur cycle. In a system where sulfur is a non-limiting nutrient, the effects would be subtle, or non-detectable in the short term, but could accumulate over long periods such as decades. Nutrient cycles are, however, related to overall ecosystem homeostasis. In mature ecosystems cycling rates are adjusted to maintain near steady states on an annual basis. Sulfur inputs significantly perturb these rates, thus perturbing the ecosystem beyond its normal homeostatic domain. Whether this perturbation is harmful or not is in large part a value judgement in the context of the benefits of generating electrical energy.

TABLE 24.3. NET INCREMENTS (POSITIVE) OR LOSSES (NEGATIVE) OF SULFUR* IN INDIVIDUAL COMPARTMENTS OVER THE 336-DAY PERIOD IN THE CONTROL AND THE 10 PPHM SO_2 SIMULATIONS†

Compartment	Control	10 pphm SO_2
Shoots	0.001	0.025
Standing dead	0.026	0.312
Roots	-0.218	-0.104
Metabolic litter	-0.176	-0.164
Structural litter	0.090	0.109
Sulfate	0.220	1.497
Reduced inorganic	0.137	0.171
Grazing nematodes	0.003	0.003
Microbes	-0.220	-0.216
Saprophagic nematodes	-0.001	-0.001
Humads	0.188	0.429
Resistant organic	-0.042	-0.011
Atmosphere	0.000	-2.030
Total reapportioned	0.661	2.536

* g S · $^{-2}$.

† Total reapportionment among compartments is the sum of either all losses or all gains and is therefore a measure of system equilibrium.

REFERENCES

Almqvist, E. 1974. An Analysis of Global Air Pollution. Ambio, 3:161-167.

Bennet, J. H., A. C. Hill, and D. M. Gates. 1973. A Model for Gaseous Pollutant Sorption by Leaves. Air Pollut. Cont. Assoc. J., 23:957-962.

Coughenour, M. B., W. J. Parton, W, K. Lauenroth, J. L. Dodd, R. G. Woodmansee. 1979. Simulation of Sulfur Cycling in Grasslands. Ecol. Model. (accepted).

Garland, J. A., W. S. Clough, and D. Fowler. 1973. Deposition of Sulphur Dioxide on Grass. Nature, 242:256-257.

Granat, L., R. O. Hallberg, and H. Rodhe. 1976. The Global Sulphur Cycle, In: B. H. Swensson and R. Soderlund, eds. Nitrogen, Phosphorus and Sulphur-global Cycles. SCORE Report 7. Ecol. Bull. 22, Stockholm. pp. 89-134.

Hutchinson, G. E. 1948. Circular Causal Systems in Ecology. Ann. New York Acad. Sci., 50:221-246.

Inoue, E. 1963. On the Turbulent Structure of Airflow Within Crop Canopies. J. Meteorol. Soc. Japan, 41:317-325.

Kabel, R. L., R. A. O'Dell, M. Taheri, and D. D. Davis. 1976. A Preliminary Model of Gaseous Pollutant Uptake by Vegetation. Center of Air Environment Studies. Publ. No. 455-476. Pennsylvania State Univ. 96 pp.

Kellogg, W. W., R. D. Cadle, E. R. Allen, A. L. Lazrus, and E. A. Martell. 1972. The Sulfur Cycle. Science, 175(11):587-596.

Lovelock, J. F., and L. Margulis. 1974. Atmospheric Homeostasis by and for the Biosphere: The Gaia Hypothesis. Tellus, 26:1-9.

Meidner, H., and T. A. Mansfield. 1968. Physiology of Stomata. McGraw-Hill Book Co., New York. 179 pp.

Monteith, J. L. 1963. Gas Exchange in Plant Communities. Chaper 7. In: L. T. Evans, ed. Environmental Control of Plant Growth. Academic Press, Inc., New York.

Monteith, J. L. 1965. Evaporation and Environment. Symp. Soc. Exp. Biol., Vol. 19, pp. 205-233.

Monteith, J. L. 1973. Principles of Environmental Physics. American Elsevier Publ. Co., Inc., New York. 241 pp.

Moss, M. R. 1976. Biogeochemical Cycles as Integrative and Spatial Models for the Study of Environmental Pollution (The Examples of the Sulphur Cycle). Int. J. Environ. Studies, 9:209-216.

Nyborg, M., and McKinnon, Allen and Associates Ltd. 1973. Atmospheric Sulphur Dioxide: Effects on the pH and Sulphur Content of Rain and Snow; Addition of Sulphur to Surface Waters, Soil, and Crops; and Acidification of Soils. Proc. Workshop on Sulphur Gas Research in Alberta. Infor. Rep. NOR-X-72, Northern Forest Research Centre, Edmonton. pp. 79-97.

Odum, E. P. 1969. The Strategy of Ecosystem Development. Science, 164:262-270.

Payrissat, M. 1974. The Cycle of Sulphur Dioxide in the Atmosphere. Eurospectra, 13(1):16-22.

Payrissat, M., and S. Beilke. 1975. Laboratory Measurements of the Uptake of Sulphur Dioxide by Different European Soils. Atmos. Environ., 9:211-217.

Redman, R. E. 1973. Plant Water Relationships. Tech. Rep. No. 29. Canadian Committee for the International Biological Program, Matador Project. 84 pp.

Ripley, E., and B. Saugier. 1975. Energy and Mass Exchange of a Native Grassland in Saskatchewan, In: D. A. deVries and N. H. Afgan, eds. Heat and Mass Transfer in the Biosphere. Part 1. Transfer Processes in the Plant Environment. John Wiley and Sons, Inc., New York. pp. 311-325.

Seim, E. 1970. Sulfur Dioxide Absorption by Soil. Ph.D. Diss. Univ. Minnesota. 138 pp.

Shepard, J. G. 1974. Measurements of the Direct Deposition of Sulphur Dioxide onto Grass and Water by the Profile Method. Atmos. Environ., 8:69-74.

Tukey, H. B., Jr. 1966. Leaching of Metabolites from Above-ground Plant Parts and its Implications. Bull. Torey Bot. Club, 93:385-401.

Unsworth, M. H., P. V. Biscoe, and V. Black. 1976. Analysis of Gas Exchange Between Plants and Polluted Atmospheres, In: Experimental Studies of the Biological Effects of Environmental Pollutants. Cambridge Univ. Press. pp. 5-16.

Whelpdale, D. M., and R. W. Shaw. 1974. Sulphur Dioxide Removal by Turbulent Transfer over Grass, Snow, and Water Surfaces. Tellus, 26:196-204.